

## REVIEW

of the official opponent for PhD thesis 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

**The rationale for choosing the research topic.** In today's world, we are witnessing an escalation of a number of social and environmental problems at both the regional and global levels. Among the latter, the negative effects of climate change are becoming increasingly noticeable, with various manifestations. These include an increase in the area where soil salinization has occurred. However, in the face of an acute need for food, humanity not only cannot allow the withdrawal of such lands from the agricultural system, but also considers undeveloped areas with high salt content as a reserve fund for agricultural production. Under such conditions, the issues of clarifying the features and patterns of response to salt stress of economically valuable crops, especially those that are not halophytes and for which even a slight increase in the salt content in the soil is a powerful stress, become especially important. Understanding the mechanisms that underlie the adaptation of plants of leading crops to salt stress conditions is becoming an important component of the implementation of measures to green the agrosphere, form sustainable agricultural prices and increase agricultural productivity.

This demonstrates the relevance of the topic of He Songtao's dissertation research that was intended: to establish mechanisms of response to salt stress and adaptation to it, which are implemented by representatives of the genus *Cucurbita* at different levels of organization, as well as eco-physiological aspects of the

formation of salt resistance, quantitative and qualitative characteristics of plants when using grafting technology.

To achieve this goal, it was formulated 11 logical, scientifically based tasks.

**The most significant scientific results obtained by the postgraduate personally.** According to the results of the study of the effect of salt stress caused by different concentrations of NaCl, the nature of the reaction of morphological features of *Cucurbita* genus representatives to salt stress was determined. It is shown that against the background of increased salt concentration, the size, and vitality of plants decrease, and a number of negative quality signs appear in them: yellowing of leaves, their twisting, etc. It has been established that changes in the dimensional parameters of plants against the background of salt stress can be an informative indicator in the study of the mechanism of salt resistance and the breeding of salt-resistant varieties of pumpkin.

It has been proven that salt stress affects indicators and signs related to the course of photosynthesis and water exchange of pumpkin plants, which leads to a decrease in the following values: photosynthesis rate, stomatal size, stomatal conductance, transpiration rate, as well as changes in chlorophyll content, mainly in the direction of its increase. An increase in the content of MDA (malondialdehyde) and the permeability of cell membrane structures due to their peroxidation was recorded in pumpkin under salt stress. The relative permeability of the plasma membrane also increased against the background of increasing salt concentration. It was shown that the transformations related to the metabolism of carbohydrates and amino acids in pumpkin play an important role in the complex of processes of response and adaptation to salt stress: with increasing salt concentration, the content of proline and soluble sugars increases. It was established that salt stress affects the absolute and relative indicators of the accumulation of ions of mineral substances, as well as their distribution in plant organs. According to these characteristics, Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> ions showed high individuality, which is also a result and evidence of their specific role both in the aspect of ensuring the reaction and adaptation of pumpkin plants to salt stress.

Identification and analysis of the expression of the WRKY gene family in *Cucurbita* genus representatives under salt stress conditions was carried out.

The interactions that occur between the scion and the salt-resistant pumpkin rootstock when using the technology of grafting in the cultivation of gourds were studied.

**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.

The methodological aspects of the assessment of salt stress impact assessment during ontogenesis of pumpkin have been improved and the technique of vitality analysis was used to assess the condition of pumpkin plants.

The efficient transient transformation system for the study of gene function and protein subcellular localization in pumpkin have been developed.

The further development of the theoretical and practical principles of the use of salt-tolerant pumpkin rootstock for cultivation of watermelon have been gained.

**The practical significance of the obtained results.** The materials of the dissertation were covered in scientific publications and included in the curricula of the specialties of 101 Ecology at the Sumy National Agrarian University (subjects: "Ecological Physiology of plants", "Agroecology", "Biology"), which is confirmed by a certificate.

**The rationale and reliability of the obtained scientific result.** The obtained results have a high scientific level. The author used a wide toolkit of scientific knowledge and research, which combined generally accepted methods, the latest approaches to solving a scientific problem, has proper statistical processing of results and their objective interpretation. The experimental part of the dissertation

is informatively illustrated and has a detailed visualization. The experimental data, computational results, and conclusions appear logical, concrete, and convincing.

**The main provisions of the dissertation published.** The main items of the thesis are presented in 12 scientific works: 3 articles were published in a scientific journals, included in the database of Scopus, Web of Science, 3 articles were published in professionals scientific journals of Ukraine, 6 - abstracts of reports at international scientific and practical conferences.

**The work consists of** an Introduction, three Chapters, Conclusions, the list of sources and five appendices. The materials of the dissertation are presented on 182 pages.

The content of the dissertation fully reflects the main results given in the research.

In **INTRODUCTION** (pp. 18-22) the relevance of the conducted research is briefly substantiated, the aim and tasks are formulated, the scientific novelty and practical value of the results, data on the approbation of the results at scientific events are given.

**CHAPTER 1** (pp. 23-47) provides a review of the literature, which summarizes the theoretical background and argues for the choice of research topics. It was carried out on the basis of literary sources, presented concisely, in a logical sequence and with a rather deep analytical approach.

This chapter includes four paragraphs covering eco-physiological and biochemical aspects of the response of plants to soil salinin and salt stress *Cucurbitaceae* family characterization provided; genetic aspects of salt tolerance of plants are revealed; characterized grafting of plants in the system of measures for growing of the *Cucurbitaceae* family and increasing their salt resistance.

**CHAPTER 2** (pp. 48-65) characterizes the research methodology. This chapter includes four paragraphs. The first one presents the basic design of the experiment. It is shown that in studies aimed at establishing the effect of salt stress on the morphological characteristics, vitality, and degree of damage to pumpkin plants, as well as on the exchange of organic and inorganic compounds, the flow of

leading physiological processes, the following experimental scheme was used: control (water, 0 mmol/L NaCl), low salt stress (60 mmol/L NaCl), high salt stress (120 mmol/L NaCl). Pumpkins of two varieties were used in the study: Yanzhen and Miben.

The following paragraphs highlight the methodological aspects of studying the grafting technology, as well as the physiological, biochemical, and genetic processes associated with plant response to salt stress and adaptation to it.

**CHAPTER 3** (pp. 66-129) covers the actual results of He Songtao's research and is entitled "Response and adaptation of pumpkin plants to salt stress". This chapter contains 5 paragraphs .

Chapter 3 thoroughly, at a high scientific and methodological level, highlights the impact of salt stress on the morphology and vitality of pumpkin plants, their leading physiological processes, organic matter metabolism, and ion absorption and accumulation. The results of the study of genetic processes as components of salt tolerance, as well as various aspects of the use of grafting technology and interspecific interactions in the system of measures to ensure adaptation and increasing salt tolerance are presented of plants.

**CONCLUSIONS** (pp. 130-131) logically and completely summarize the results of the conducted research. The content of the conclusions certifies that the dissertation fulfills the tasks and achieves the purpose of the work.

The list of references includes 352 sources. It presents the leading scientific works on the subject of research, which reflect the scientific achievements of researchers from different countries and a fairly wide range of time. A significant share is made up of the latest scientific works.

**The degree of validity of research results, reliability of data and their novelty.** In the course of research, a rather broad scientific program was carried out. The research was carried out at the modern level, using classical and modern methods of genetics, physiology and ecology, which allowed us to cover different levels of living organization that play a leading role in ensuring the response of representatives of the *Cucurbita* genus to salt stress and adaptation to it. The

results of the research are calculated with statistical analysis and have a correct and convincing interpretation. The conclusions of the dissertation are logically composed on the basis of the research results highlighted in the chapters of the work. The dissertation has a high level of scientific novelty. Thus, the dissertation has a high theoretical level, and its results are of significant scientific value for further research.

**Evaluation of the language and style of the dissertation.** The thesis is written in scientific English with the use of a large amount of illustrative material, which improves the understanding of experimental data. A brief summary of the research results, presented in an understandable logical sequence. The style of the dissertation meets the generally accepted requirements in ecological research.

**Correspondence of the dissertation to the specialty and the profile of the council.** The dissertation totally corresponds to the field of knowledge 10 «Natural Sciences» specialty 101 "Ecology".

**Discussion clauses and remarks to the dissertation.** Positively evaluating He Songtao's dissertation work, its important scientific-theoretical and practical significance, novelty and relevance of research, it is appropriate to make the following remarks and wishes.

1. Taking into account that the work was carried out on the basis of international cooperation between Sumy National Agrarian University and Henan University of Science and Technology, the paper should have paid attention to the comparison of soil salinity in Ukraine and China, and the relevance of the issue of adaptation of biological, ecological systems and production processes to this phenomenon for these two countries.
2. In chapter 2 (p. 51), it was necessary to describe in more detail the algorithm for calculating the vitality analysis used to assess the vitality of plants against salt stress.
3. Some of the data presented in Figure 3.2 (pp. 68, 69), Figure 3.8 (pp. 83, 84), Figure 3.9 (pp. 85, 86), Fig. 3.10 (pp. 88, 89) should have been presented in the form of tables.

4. In the conclusions, it was necessary to detail the content of paragraph 6 to a greater extent.

5. The work was carried out with extensive use of mathematical statistics methods, in particular, analysis of variance. However, the results of these calculations should have been presented in a more detailed form in the appendices.

6. The work contains some grammatical errors and shortcomings related to the formatting of the work (for example, incorrect abbreviations).

As a suggestion for the applicant, we should note that the work presents a number of developments that are important from a practical point of view, so in the future it is worth paying more attention to the implementation of the results of their research, in particular those related to the technology of cuttings and (or) the use of arbuscular mycorrhizal fungi, in farms that grow melons. This experience is also valuable for Ukraine.

**General conclusion.** The dissertation work of 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 obtaining the degree of Doctor of Philosophy in the field of knowledge 10 – «Natural Sciences» in the 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 registration 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.

Based on the mentioned above, I believe that the author of the dissertation, He Songtao, deserves to be awarded the scientific degree of Doctor of Philosophy in the specialty of 101 – «Ecology» of the field of knowledge 10 – «Natural Sciences».

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