REVIEW

of the official opponent **Mykola MARENYCH**

for **Qiaoyan Chen** dissertation on the topic **«Breeding and genetic bases of winter wheat ear traits»**,

applied for the degree of Doctor of Philosophy

from the field of knowledge 20 Agricultural sciences and food

in the specialty 201 Agronomy

Relevance of the topic and the obtained results.

The relevance of the dissertation research is determined by the global importance of winter wheat for human nutrition. In the introduction to the dissertation, the author emphasizes the significance of this crop from a demographic perspective and the application of new genetic approaches, such as molecular marker mapping, molecular biology, and the further development of biometrics and biological statistics. The main advantage of these methods is the potential for significantly reducing the breeding process through precise genetic management of the inheritance and expression of economically valuable traits..

Modern breeding increasingly resorts to another promising method - the creation of almost-isogenic lines. This method allows combining a wide range of economically valuable traits and productivity elements in wheat plants, ensuring dynamic yield growth. In this context, specific tasks arise to study the patterns of expression of major quantitative traits, such as the number and weight of grains, and the possibilities of genetic management of these traits.

It should be noted that the application of innovative plant breeding methods requires substantial theoretical analysis, the identification of patterns in the expression and formation of productivity traits, and extensive experimental research. Therefore, the dissertation is done on a highly relevant and interesting topic.

The most significant scientific results obtained by the researcher personally consist in the independent planning and conducting research. The author personally analysed and summarized the theoretical material relevant to the chosen scientific topic. As a result, a program of experimental research was developed and implemented, which included a cycle of field and laboratory research. Field research was conducted during 2018-2019. The author carried out laboratory and biometric research of traits of the number of grains and the weight of 1000 grains. The doctoral student has mastered modern methods of molecular biology and genetic analysis. A significant volume of biometric measurements at the molecular level was also carried out, in particular microRNA nucleotides and their sequences.

The scientific novelty of the obtained results lies in the application of molecular biology and genetic analysis methods to achieve specific breeding goals for winter wheat. The genetic mechanisms of the formation and inheritance of important traits such as ear productivity and the weight of 1000 grains were studied. During the dissertation research, the author used quite modern laboratory equipment,

which in turn allowed for the investigation of important aspects at the molecular level related to the most economically valuable traits. The results obtained on the genetic determinants of yield formation in wheat, as a complex indicator, create certain prerequisites for enriching the theoretical foundations of wheat breeding for productivity and detailing the mechanisms of grain formation and development.

The practical significance of the obtained results lies in a certain optimization of the breeding process. The mechanism of genetic control of productivity traits, in particular, the number of grains in an ear, has been established, which creates prerequisites for the study of other genetic processes. Based on the conducted research, a map of almost one and a half hundred genetic loci in 19 chromosomes was created. During the research, 79 new microRNAs were identified. Based on the identification of genes and markers of economically valuable traits, a conclusion was made about a more effective breeding of wheat plants with the desired traits, which has a very real prospect for implementation at the phenotypic level, which means obtaining a higher yield.

Justification and reliability of the obtained scientific results.

The scientific-methodical and scientific-technical level of the conducted research is quite high. Application of methods of molecular biology and genetic analysis in combination with biometric definitions contributed to the establishment of important regularities in the formation of economic and valuable traits. The role of microRNA in the mechanisms of grain formation was established, which allowed the author to make an assumption about the possibility of molecular ways of improving the wheat breeding process.

The scientific results were obtained during the implementation of a complex research program, which involved the use of field experiments and the use of modern molecular biological methods. Experimental material for research was selected randomly, which made it possible to form a representative sample for conducting experiments.

A wide range of research methods, modern techniques and modern biometric assessment methods are used in the research. As a result of the statistical processing of the results of the experiment, it was established that the phenotypic manifestations of the parental forms according to the number of ears in the ear and the weight of 1000 grains could be used for their breeding for crossing. These trait

s had high heritability and were characterized by a strong correlation dependence. A gene locus has been established that controls almost 11.5% of the phenotypic manifestation of the 1000-grain mass trait and can increase this indicator. Nine epistatic loci were also established, which are responsible for the formation of the number of grains in an ear and the weight of 1000 grains. It was also established that epistatic loci of the parental form could control almost 42% of the total phenotypic variation.

The main results and provisions of the research are published in two publications entered into the Scopus scientometric database, three specialized scientific publications of Ukraine and seven conference materials.

In the **introduction**, there are quite specific, albeit brief, details about the relevance of the chosen research direction, its purpose, scientific novelty, and the specified research methods. The main goal of the research was to confirm the possibilities of marker breedingn and its prospects for practical application. The research methods are also mentioned, along with the practical value of the obtained results. The author's personal contribution is described as a thorough analysis of the theoretical material, personal execution of the research, and processing of the obtained results.

Chapter 1 consists of several subchapters where the author attempted to adhere to a logical presentation of theoretical material. In clause 1.1, the author describes the localization of quantitative traits related to grain number in the ear and 1000-grain weight. The results of using DNA molecular markers and their role in the wheat breeeding process are detailed. The author emphasizes the importance of selecting parental pairs that do not exhibit significant differences. Throughout the literature narrative, the feasibility and benefits of using second-generation hybrid populations and the role of backcrossing are explored, which allows for more precise gene localization. The chapter includes a relatively detailed description of gene mapping methods. Additionally, genes responsible for grain number in the ear, 1000-grain weight, and their interactions with other traits and genes are described in detail. The advantages of using genetic markers in practical breeding are highlighted, significantly reducing the breeding process. Based on the literature review, the most effective method for increasing yield in regional breeding is identified as increasing the number of grains in the ear.

Chapter 2 contains a description of the source material - the two varieties that were studied and a brief description of the experimental program. Methods of DNA extraction and analysis of molecular markers are described step by step and in sufficient detail. This section also lists the equipment and software used during the laboratory cycle of research. This section also contains the first results obtained in the course of research - analysis of phenotypic data, loci of the number and weight of 1000 grains, and genetic linkage maps.

As follows from the description of the research methodology, they were performed at a high technical level, which made it possible to obtain representative results. This applies to the determination of molecular markers, DNA extraction, genetic linkage mapping, etc. It was established that the number of grains in an ear depended on the role of the mother form, and the mass of 1000 grains depended on the parent form - the Chinese variety. Thus, the author determined that both traits have a high rate of heredity. The author established chromosome loci that control 4.92–21% of the manifestations of the phenotypic reaction of the number of grains in an ear and the weight of 1000 grains. It was established that the effect of epistasis

has a greater influence on the phenotypic manifestation of the trait - 41.9%. The chapter ends with conclusions regarding the obtained results.

Chapter 3 presents the research methodology and literature review related to the study of microRNAs, as well as the research results. It has been established that species specificity is reflected by the length of mRNA. In the course of research, 79 new microRNAs were identified and the reliability of sequencing based on mRNA expression patterns was established. The functionality of these substances involves recombination and recombination 11.9%, transcription 8.73% and protein modification – 5.56%, protective mechanisms and transport function – 2.38–3.17%. Having determined the functionality of mRNA in this way, the author concludes about the possibility of the existence of an important role of mRNA in the growth and development of wheat plants. The role of mRNA in the mitotic cell cycle, nuclear division, cell morphogenesis, cell differentiation and the development of wheat tissues and traits has been established.

The **conclusions** appear detailed but lack structure. They contain both general interpretations of the role of micro-RNAs for the development of wheat grain and to clarify their role in molecular mechanisms, as well as specific information on the importance and functions of known and new compounds (micro-RNAs) in the formation of plant organs at the cellular level.

Evaluation of the language and style of the dissertation: The dissertation, written in English and heavily laden with scientific terminology, gives the impression of extensive effort. However, its chapters often do not correlate well with the subsequent material presented. There is a desire for a greater number of illustrations, which are currently difficult to read. The obtained results are voluminous but lack argumentation, are presented in a not entirely logical sequence, and lack interpretation. No mathematical evaluation criteria are provided.

Compliance of the dissertation with the specified specialty and requirements. The topic of the dissertation corresponds to the aspects of genetics, molecular and cellular biology and wheat breeding. Unfortunately, the dissertation lacks sufficient information on the phenotypic expression of quantitative traits, which complicates its assessment from a breeding perspective.

Discussion clauses and remarks to the dissertation. The thesis gives a contradictory impression. On one hand, the relevance of the topic is indisputable, and the scientific and technical toolkit selected for addressing the research objectives allows for discussing the representativeness of the obtained results and the further prospects of research and scientific implementation. On the other hand, the design, logical coherence, and informativeness of presentation, as well as the interpretation of certain points, require detailed clarification. Primarily, this concerns the following points.

1. On page 24, the dissertation's connection to scientific topics is described in one word.

2. When discussing genetic differences between parental forms, the author

starts the analysis of literature sources from tomatoes, which shifts the research focus.

3. It would be advisable to place Table 1.1 on page 35.

4. Field research was conducted during 2018-2019, but from an agronomic standpoint, they lasted only one or two years. Therefore, it was necessary to describe in detail the methodology that allowed obtaining representative data, which would also enable formulating representative results.

5. It is unclear from the research methodology why second-generation hybrids were chosen. It is mentioned only once that they can provide the fullest spectrum of genotypes, but this is insufficient. Why were these specific parental forms chosen? Why were only the specified productivity traits selected and not others? What are the correlations between productivity elements and morphological traits? Questions arise, such as how all of this relates to yield through correlations.

6. In Chapter 3, the presentation of the research methodology begins again, although this should have been done in the previous chapter.

7. The presentation of material appears as overly concise statements of facts without argumentation and formulation of preliminary conclusions that would allow for the formulation of main conclusions.

8. Figures in the dissertation are presented in a small scale, making it impossible to read most of them, and the reader is forced to rely solely on the author's interpretation in their descriptions.

9. According to the opponent's opinion, the conclusions poorly correspond to the content of the chapters themselves and hardly correspond to the purpose, objectives of the research program, even the keywords listed in the dissertation abstract, its relevance, and the scientific novelty of the research.

10. Point 2 of the conclusions discusses the heritability of quantitative traits (likely grain number in an ear and 1000-grain weight), but this is not a scientific novelty.

11. In Point 3 of the conclusions, it is stated that there is a direct correlation of 0.953 between the number of ears (or grains?) and the 1000-grain weight, but it is unclear why there is a direct and almost functional correlation between traits that genetically have an inverse relationship according to the author's research.

12. A significant number of conclusions, specifically points 4, 5, 7, 9, 10, 18, 19, 22, and others, simply state individual narrow aspects of the dissertation and do not correspond to the conclusions in essence.

13. Point 20 of the conclusions does not explain what exactly caused the increase in grain weight, a metric that is not even discussed in the dissertation. Is it referring to the 1000-grain weight?

14. The recommendations based on the research results do not correspond in essence to the concept of recommendations themselves and do not arise from the content, purpose, and objectives of the dissertation research. They merely describe the research purpose and possibilities once again.

The mentioned comments and questions require detailed explanations and answers, as well as the substantiation of points during the public defense.

General conclusion. Dissertation work by Qiaoyan Chen titled "Breeding and genetic bases of winter wheat ear traits", submitted for public defence for obtaining the degree of Doctor of Philosophy in the field of knowledge 20 - Agricultural sciences and food in the specialty 201 - Agronomy by its relevance, scientific and methodical level, obtained and the published results meet the requirements of the Order of the Ministry of Education and Science of Ukraine No. 40 dated January 12, 2017, "On Approval of the Requirements for the Dissertation Formatting," and the Resolution of the Cabinet of Ministers of Ukraine dated January 12, 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 a Higher Education Institution, Scientific Institution on Awarding the Degree of Doctor of Philosophy," as amended by the Resolution of the Cabinet of Ministers No. 341 dated March 21, 2022. The author of the dissertation, Qiaoyan Chen, deserves to be awarded the degree of Doctor of Philosophy in specialty 201 - Agronomy.

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Ідентифікаційний код 00493014