REVIEW

of the Official Opponent ***Anna Kryvenko,***
for PhD Thesis ***Zhu Yinghui***
«Insights into profiling of resistance mechanism of alfalfa to atrazine», Submitted for a Scientific Degree of a Doctor of Philosophy Field of Study: 20 – Agricultural Sciences and Food Specialty 202 – Plant Protection and Quarantine

The most significant scientific results obtained by the postgraduate personally. The obtained data on the harmfulness and mechanisms of atrazine resistance of different alfalfa varieties will further provide a theoretical basis for breeding varieties resistant to atrazine. In field experiments, it was confirmed that the absorption of atrazine by resistant and susceptible alfalfa showed an increasing trend, and the degradation of atrazine by the soil showed a decreasing trend. Thus, this provided a theoretical basis for the scientific and rational use of atrazine.

In a greenhouse climate chamber, resistant SF and sensitive J2 alfalfa varieties were screened by measuring changes in fresh weight inhibition rate.

Using SF and J2 alfalfa as research objects, the P450s inhibitor malathion was studied for its involvement in the metabolic effects of SF on atrazine. Next, it was found that under different concentrations of atrazine stress, the plant height, fresh weight, plant dry weight, root dry weight, photosynthetic characteristics, chloroplast fluorescence parameters, chlorophyll content, and MDA content of SF and J2 were affected to varying degrees, and the differences between the two were significant.

Transcriptome sequencing revealed significant genetic differences between SF and J2 plants. Field experiments conducted at the Henan Academy of Agricultural Sciences in China from 2022 to 2024 once again demonstrated that the residual levels of atrazine in SF and J2 alfalfa were different, and the differences between the two were significant.

The rationale and reliability of the obtained scientific results. The dissertation has a high level of scientific research has a high level of scientific research and proposes agricultural techniques for planting and cultivating alfalfa varieties resistant to atrazine and atrazine under plain conditions in Henan Province, China. The dissertation work adopts recognized scientific work and analytical methods, which can obtain reliable experimental data on variable differences, and based on this, formulate scientific conclusions and production recommendations.

According to the cultivation technology of alfalfa under plain conditions in Henan Province, China, it has economic and energy benefits, indicating a modern comprehensive method for conducting scientific research.

The main provisions of the thesis are presented in 7 scientific works, of which 4 articles (three were published in professional publications of Ukraine and 1 - publications included in the international databases WoS and Scopus). Also, the work has been tested quite well, and the research results have been highlighted in three theses of reports at international scientific conferences. The materials of the articles and theses of the reports reflect the main provisions and conclusions of the dissertation work.

In the introduction provided data on the relevance of scientific research, formulated goals and tasks, scientific novelty and practical value of the dissertation.

In the first section provided data on soil and climatic conditions and weather indicators during the years of field research in the Henan, China. Data on experimental schemes, the program and research methodology are presented.

In the second section screened the scientific research results of resistant SF and sensitive J2 alfalfa varieties resistant to atrazine. The results

indicated that under the stress of atrazine, SF with the highest IC_{50} and J2 alfalfa with the lowest were selected, indicating that SF developed the highest resistance to atrazine and J2 was the most sensitive to atrazine.

In the third section, by comparing the synergistic effects of malathion and NBD-CI on atrazine in different alfalfa varieties, it was shown that P450s were involved in the development of resistance to SF metabolism, while NBD-CI was not involved. From this, it can be seen that the resistance of SF and J2 to atrazine was related to P450s and not to GSTs.

SF and J2 both suffered varying degrees of damage under atrazine stress, but the differences between the two were significant. The plant height, root length, shoot dry weight and root dry weight of SF and J2 were all damaged. The indicated differences in resistance between SF and J2 to atrazine.

Under the stress of atrazine, the Pn of SF and J2 alfalfa, Gs and Tr decreased, while Ci increased, and the difference between the two was significant. Alfalfa reduced the damage of atrazine to its leaf photosynthetic system by enhancing respiration.

Under the stress of atrazine, the Fv/Fm, Fv/Fo, Y (II), ETR, and qP of SF and J2 decreased, while NPQ increased, and the difference between the two was significant. This indicated that atrazine stress caused damage to the PSII reaction centers of SF and J2, thereby inhibiting plant photosynthesis.

The residue of atrazine reduced the content of chlorophyll a and chlorophyll b in SF and J2 alfalfa leaves, and the difference between the two was significant. J2 was more sensitive to atrazine than SF.

Atrazine residue increased the MDA content in SF and J2 alfalfa and the significant difference between the two indicated that J2 was more susceptible to damage under atrazine stress than SF.

In the fourth section. The absorption and metabolic residue detection of SF and J2 by UPLC-MS/MS showed that compared to SF, J2 had faster absorption but slower metabolism, and the difference between the two was significant. SF was more resistant to atrazine than J2 alfalfa.

In the fifth section verified the reliability of transcriptome sequencing data from a molecular biology perspective. The clean bases of each sample reached 6.34 Gb or above, with base error rates less than 0.10%. The Q20 base percentage was above 97.8%, and the Q30 base percentage was above 93.48%, indicating that the sequencing data met the requirements.

Through GO and KEGG analysis, key pathway genes involved in photosynthetic carbon utilization and metabolism, amino acid synthesis, and UDP galactosyltransferase activity in SF and J2 were identified, indicating that these genes were one of the main reasons for SF and J2 to produce anti atrazine.

In the sixth section. In field experiments conducted between 2022-2023 and 2023-2024, the residue of atrazine in soil showed a trend of first decreasing and then stabilizing, while the residue in SF and J2 showed a trend of first increasing and then decreasing, with significant differences. It indicated that atrazine will remain in the soil and alfalfa.

The conclusions and **recommendations for production**. For the screened resistant SF and sensitive J2 alfalfa as research objects, the genes that produce resistance to the atrazine were identified through molecular mechanisms, and laboratory and field cultivation were carried out. This provided a theoretical basis and practical solutions for breeding varieties in different regions of agricultural production.

Compliance of the dissertation with the specified specialty and requirements. The dissertation fully corresponds to the field of knowledge

20 “Agrarian sciences and food” specialty 202 “Plant Protection and Quarantine”.

Discussion points and comments. Despite the high level of research conducted, some provisions of the work are debatable and have grounds for comments.

1. Why was the determination of atrazine residues in soil and plants carried out only during two growing seasons of alfalfa?
2. When covering the issue of the distribution and harmfulness of atrazine in Chapter 1, literary sources for the last ten years were not analyzed, most of the references refer to the early 2000s.
3. Individual chapters of the dissertation are divided into a large number of subsections, which complicates the perception of information.
4. When studying the mechanisms of resistance, most of the research was related to the impact on the photosynthetic activity of alfalfa.

General conclusion. The dissertation work of Zhu Yinghui intitled «Insights into profiling of resistance mechanism of alfalfa to atrazine», which was submitted for defense to the specialized academic council for obtaining the degree of Doctor of Philosophy in the field of knowledge 20 -“Agricultural sciences and food” in the specialty 202 - “Plant Protection and Quarantine” in terms of its relevance, scientific and theoretical level, main results of validity, main provisions and results published in professional publications, and novelty of the setting and practical significance meets the requirements of the order of the Ministry of Education and Science of Ukraine No.40 dated January 12, 2017 “On approval of requirements for registration dissertation” and the Decree 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 the higher educational institution, scientific institution on awarding the degree of "Doctor of Philosophy"with amendments made according to the Resolution of the Cabinet of Ministers No. 341 dated 03.21.2022. The PhD candidate Zhu Yinghui deserves to be awarded the scientific degree of Doctor of Philosophy in specialty 202 - "Plant Protection and Quarantine".

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Підпис Кривенко Анни засвідчую!
ст. інспектор з кадрів [illegible] Ірина Ткаченко