



## REVIEW

on a PhD thesis for obtaining an educational and scientific degree "Doctor (PhD)" in: the field of higher education 6. Agricultural Sciences and Veterinary Medicine, professional field 6.1 Crop production, scientific specialty Crop production.

**Author of the dissertation:** Hristina Atanasova Nedeva PhD student at the Department of Crop Science at the Agricultural University, Plovdiv

**Topic of the dissertation:** EFFECT OF NITROGEN FERTILIZATION AND HARVEST TIMING ON THE PRODUCTIVITY AND QUALITY OF GREEN BIOMASS FROM TRITICALE USED FOR ENERGY PURPOSES.

**Reviewer:** Prof. Dr Hristofor Kirchev Kirchev, Agricultural University, a field of higher education 6. Agricultural sciences and veterinary medicine, professional field 6.1 Crop production, scientific specialty Crop production.

Appointed a member of the scientific jury by order № RD-16-1100/ 27.10.2022 by the Rector of AU.

### 1. Brief presentation of the applicant.

Hristina Atanasova Nedeva was born on May 12, 1977. During the period 1996-2001, she studied at the Agricultural University of Plovdiv and obtained a master's degree in Agronomy. Since 2007, he has been working at the Experimental Station for Irrigated Agriculture in the city of Pazardzhik (JV "OSPZ - Pazardzhik" DP) as a research assistant with the task of carrying out varietal comparison and demonstration experiments. Since 2014, she has been working at "River Garden" EOOD as an agronomist, where he organizes and manages the production of the produce. In 2021, she was again appointed to SP "OSPZ - Pazardzhik" DP as an assistant in varietal agrotechnics. In 2022, she was enrolled as a PhD student of independent training in the Department of Crop Science at the Agricultural University - Plovdiv. In a short period, she completed her studies and was dismissed with the right to defence in the same year.

### 2. Relevance of the problem.

Biofuels are liquid or gaseous fuels produced from biomass consisting of plants or plant-based materials. They are used as a substitute for fossil fuels, mainly in the transport sector. First-generation biofuels are produced from food crops, e.g. corn, sugarcane and soybeans. Second-generation biofuels are produced from feedstocks that are not normally obtained from food crops and are not suitable for human consumption. These include used cooking fat and waste from agriculture and forestry.

The most used raw materials for energy production from plants are corn, sugar cane, soybeans and potatoes. Recently, alternatives to these crops have been sought, since they are one of the main food plants and their use for energy purposes will lead

to a disruption of the nutritional balance of the ever-increasing population of the planet. Such an alternative is the man-made triticale culture, combining in itself the high productive potential of biomass and its high energy value with the low cost of cultivation due to the elimination of some costs in triticale agrotechnics.

In the last more than 20 years, triticale research in Bulgaria has been almost entirely focused on grain. There are very few studies on the cultivation of triticale for green mass, and it is one of the most productive grain-forage crops in this direction.

### **3. Purpose, tasks, hypotheses and research methods.**

The present study was conducted to determine the effect of applying different nitrogen fertilizer rates and harvest phases on the yield and quality of triticale green mass intended for biogas production.

To fulfil the set goal, five tasks have been set as follows:

1. To study the influence of different rates of nitrogen fertilization and harvesting phases on the growth and development of triticale grown for green mass.

2. To study the influence of different rates of nitrogen fertilization and harvesting phases on physiological parameters of triticale grown for green mass.

3. To study the influence of different rates of nitrogen fertilization and harvesting phases on the structural elements forming green biomass.

4. To study the influence of different rates of nitrogen fertilization and harvesting phases on the yield of green biomass.

5. To study the influence of different rates of nitrogen fertilization and harvesting phases on the quality of the obtained production intended for biofuel.

To achieve the goal and tasks of the study, three years of field experiments were undertaken.

The field experiments were carried out at the Department of Crop Science, Agrarian University - Plovdiv, after a predecessor of rapeseed. The experiments were carried out using the method of split plots in 4 repetitions with the size of the experimental plot 20 m<sup>2</sup>. The following factors were tested:

Factor A - triticale varieties:

A1 – variety Musala

A2 – variety Attila

Factor B - rates of nitrogen fertilization on background P<sub>15</sub> K<sub>10</sub>:

B1 – N<sub>0</sub>

B2 – N<sub>12</sub>

B3 – N<sub>16</sub>

B4 – N<sub>20</sub>

B5 – N<sub>24</sub>

Factor C - harvesting phases:

C1 – spike emergence

C2 – milk maturity

Factor D - Conditions of the year

D1 – 2013/2014

D2 – 2014/2015

D3 – 2015/2016

The following indicators were studied:

- Stages of development - defined according to the BBCH scale for cereals - emergence, third leaf, tillering, stem elongation, spike emergence, milk maturity.
- Structural elements of the yield - determined before harvesting. 50 plants of each variant were analyzed in the spike emergence and milk maturity at harvesting, in fresh condition, and the plant height components were determined; a number of plants per m<sup>2</sup> - when harvesting; common tillering; number of spikes per 1 m<sup>2</sup>; spike length - from the base to the tip; number of leaves per plant; thickness of stems; mass of one plant; mass of stems of one plant; leaf mass of one plant; spike mass of one plant; percentage ratio: stems: leaves: spike of one plant.
- Yield of green mass - determined by weighing the biomass in the stage of stem elongation and milk maturity for each variant, by replications.
- Physiological indicators were performed in the laboratory at the Department of Physiology and Biochemistry of Plants, where the leaf area in the stages of spike emergence and milk maturity, the intensity of transpiration, stomatal conductance and the number of plastid pigments were determined. The physiological parameters of net photosynthesis rate, transpiration intensity and stomatal conductance were determined with a portable photosynthetic system LCA-4. In the laboratory of the department of "Physiology and Biochemistry" at the Agrarian University - Plovdiv, the analyzes were also made for the number of plastid pigments - chlorophyll A, chlorophyll B, chlorophyll (A+B) and carotene.
- The chemical composition of the green mass was investigated in two phases (stem elongation and milk maturity), determining the content of dry matter, crude protein, crude fat, crude fibre, non-nitrogen extractive substances, sugars + starch.
- Soil indicators - the content of nitrogen, phosphorus and potassium, pH of the soil - before sowing and after harvesting the predecessor.
- For the statistical processing of the reported experimental data, the dispersion analysis method was used, using Biostat, SPSS for windows, v 9.00; Duncan's Multiple Range Test.

#### **4. Visualization and presentation of the obtained results.**

The scientific work submitted for review contains 169 pages, including 47 tables and 6 figures. The list of cited literature contains a total of 230 literary sources, of which 79 are in Cyrillic and the rest are in Latin.

The PhD thesis contains all sections generally accepted for this type of presentation, namely: Contents - 2 pages; Introduction – 2 pages; Literature review – 32 pages; Purpose and tasks of the research - 1 page; Material and methods – 6 pages; Soil and climate characteristics – 8 pages; Results and discussion – 95 pages; Conclusions – 3 pages; Contributions – 2 pages and References – 19 pages.

The introduction presents the triticale specie in general, its advantages, and the main producers of triticale in the world, and introduces the reader to the subject of the new development strategy of the European Union, part of which is the "Green Deal", in connection with the search for alternative methods for the production of green energy, where crops such as triticale are finding more and

more space.

The literature review is divided into subsections describing studies on the use of renewable raw materials of plant origin for the production of biofuels, extraction of methane and biogas from various crops, creation, development and use of triticale for green biomass in the world and Bulgaria, the quality of triticale green mass and other crops suitable for biogas and methane production and the effect of fertilization on the productivity and quality of triticale green mass.

The soil-climatic characteristics include the general climatic characteristics of the area of the experiment - the city of Plovdiv, the soil characteristics of the field, as well as an analysis of the agro-meteorological conditions during the study period.

## **5. Discussion of results and literature used.**

As a result of the experimental activity, the obtained data are described and discussed in the Results and discussion section. The section is divided into 5 subsections.

The first subsection describes the influence of nitrogen fertilization and harvesting phases on the phenological development of triticale for green mass. The dates of occurrence of the emergence phenophases (BBCH 11) are reflected; third leave (BBC13); tillering (BBCH 21); stem elongation (BBCH 43); spike emergence (BBCH 57) and milk maturity (BBCH 75). Using the appropriate code on the BBCH scale gives a clear idea of the exact time of the phenophase reading. The duration of the interphase periods and the sum of the active temperatures by phase for the two varieties of triticale were recorded.

In the next subsection, the influence of nitrogen fertilization and harvesting phases on the physiological parameters of triticale for green mass is investigated. Analyzes were made of the changes in the leaf area of a flag leaf from a central tiller in the stage of spike emergence and milk maturity, the parameters of leaf gas exchange and the amount of plastid pigments.

The following subsection describes the productivity of triticale for green mass. The influence of nitrogen fertilization and harvesting phases on the green mass yield of triticale was studied. The yields of green mass in the stage of spike emergence and milk maturity for the two varieties are presented in tables with the corresponding comments. A separate table presents the influence of factors on yield variation. The effect of nitrogen fertilization of 1 kg of nitrogen is described, depending on nitrogen fertilizer rates and harvest phases. This study answers the question of what increase in yields is obtained as a result of the application of the nutrient. Basic parameters of the crop are presented, such as total and productive tillering and number of spike-bearing stems, as well as the biometric parameters plant height, stem thickness, number of leaves per plant, number of spikes per plant, and spike length. The influence of nitrogen fertilization and harvesting phases on the morphological structure of green plants is described - the distribution of plant organs (stems, leaves and spikes) concerning the total biomass.

In the subsection triticale productivity, dry mass and silage, the influence of nitrogen fertilization and harvesting phases on the yield and dry matter content of the



two triticale varieties are described. This subsection is also the main part of the study, namely the use of triticale for energy purposes - energy efficiency and the influence of nitrogen fertilization and harvesting phases on the production of biogas and methane. It is specified that the theoretical yield of biogas and methane was calculated based on the average yields of silage obtained from the green mass.

In the last subsection, the chemical composition of triticale green mass was studied. The influence of nitrogen fertilization and harvesting phases on the content of chemical indicators in the green mass - crude protein, crude fibres, crude fats, nitrogen-free extractive substances, sugars and starches, as well as the yields of the relevant organic components - is described.

Based on the obtained and analyzed results, the PhD student formulates 15 conclusions, which summarize the research in abbreviated form.

## **6. PhD thesis contributions.**

The research results make it possible to form a total of 10 contributions, divided into 5 scientific-theoretical and 5 scientific-applied, as follows:

### **Scientific and theoretical contributions**

1. Varietal differences in the phenological development of the tested varieties of triticale, Musala and Atila, grown for green mass, were found. The duration and the necessary temperature sum for the interphase periods and their vegetation period under different weather conditions during the years of research for the conditions of the Plovdiv region were determined.
2. The physiological characteristics of triticale in Attila and Musala varieties for green mass in the Plovdiv region and their change under the influence of nitrogen fertilization and harvesting phases were established.
3. Atila variety was found to be superior to the Musala variety in nitrogen assimilation efficiency, being more energy efficient, obtaining higher yields with input nitrogen consumption of 1 kg.
4. The chemical composition and the yield of the nutritional elements of the biomass in the tested varieties and the influence of nitrogen fertilization on them were determined.
5. Theoretically, the yields of silage, biogas and methane were calculated for the chosen variants of the two varieties of triticale, Musala and Atila.

### **Scientific and applied contributions**

1. The genotypic specificity of two modern Bulgarian triticale varieties has been established for the level of nitrogen fertilization and the phases of harvesting for green mass under the conditions of Central South Bulgaria.
2. It was established that in heavy rainfall during the grading period accompanied by strong winds, the Musala variety showed a lower resistance to lodging compared to the Atila variety.
3. It has been proven that the Musala variety is more suitable in terms of yields of green mass in drier years for the region of Central South Bulgaria.
4. A stable tendency to increase yields of green and dry mass is outlined when nitrogen fertilizer rates are applied. The highest yields are obtained when triticale varieties are fertilized with a nitrogen rate of 20 kg/da. The use of a high nitrogen rate of 24 kg/da does not positively affect yields.

5. Under the influence of the changing weather conditions, a varietal response was established regarding the quality of the green mass. The more pronounced effect of nitrogen fertilization on the quality of triticale green mass compared to the influence of the variety is confirmed.

## 7. Critical notes and questions.

- The author sets himself the ambitious goal of exploring the use of green triticale biomass for energy purposes. The literature review also introduces to a large extent in this direction. However, in the actual part of the study (results and discussion section), the research in this direction is only hinted at and presented rather sparsely.

- The material and methods section lacks a description of the methodology used to determine the content of methane and biogas.

- There are spelling and technical errors.

- No. 12 is omitted in the numbering of the tables, and source No. 76 in the literature.

- The term "mass" should be used instead of weight.

- All Latin names must be written in *italics*.

Despite the indicated omissions, this is a large-scale study of triticale grown for green mass and the notes made do not detract from the contributions of the dissertation.

## 8. Published articles and citations.

According to the minimum scientometric requirements specified in the Regulations for the Application of the Law on the Development of the Academic Staff, 3 publications related to the dissertation are indicated, which fully cover and even exceed the required number of points.

The presented abstract reflects objectively the structure and content of the dissertation work.

## CONCLUSION:

Based on the research methods learned and applied by the PhD student, the experiments carried out, and the summaries and conclusions are drawn, I believe that the presented PhD thesis meets the requirements of the Law and the Regulations of the Agricultural University for its application, which gives me a reason to evaluate it **POSITIVE**.

I would like to suggest to the esteemed Scientific Jury to vote positively and to award Hristina Atanasova Nedeva the educational and scientific degree "**Doctor**" in the scientific specialty of Crop production.

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Plovdiv

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