AGRICULTURAL UNIVERSITY PLOVDIV

Agronomy Faculty

DEPARTMENT OF CROP SCIENCE

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Effect of nitrogen fertilization and harvest time on productivity and quality of triticale green biomass used for energy purposes

A V T O R E F E R A T

FOR THE AWARD OF THE DEGREE OF DOCTOR

IN THE SCIENTIFIC SPECIALTY "CROP TECHNOLOGY"

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Plovdiv,

2022

The research was carried out during 2014-2016 at the Agronomy Faculty of Agricultural University, Plovdiv.

The thesis is 169 pages and contains 48 tables and 6 figures. The cited literature includes 231 sources.

The thesis work has been discussed and directed for defense by the council of the "Crop Science" department at the Agronomy Faculty of the Agricultural University - Plovdiv.

The official defense of the thesis will take place on ………from……….hours at a meeting of the Specialized Scientific Jury at the Agricultural University of Plovdiv with members:

Internal Members:

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1. INTRODUCTION

In connection with world trends, the new development strategy of the European Union, part of which is the "Green Deal," and in connection with the use of alternative methods for the production of green energy, crops such as triticale are finding more and more space.

The great potential of the triticale in this direction is due to the unique combination of the good qualities of the species from which it originates, namely - rye and wheat. The crop fits very well in the circular bioeconomy, which is increasingly being imposed recently, where the main share of crop production is the production of raw materials.

Our country's average yields of green mass, depending on the variety and climatic factors of the respective region, ranging from 3 to 7 t/day, is equivalent to 1000-1200 kg/day of dry matter.

Apart from the versatile use of triticale so far, there has been an increased interest in this crop and biogas production in recent years. This is a prerequisite for a continuous increase in areas, yields, and production in the world and our country.

As a result, the total world area increased from 12,500,000 da in 1990 to 24,949,820 da in 2000 to 38,076,293 da in 2019.

The largest producers of triticale in the world are Poland, Germany, France, China, Hungary, Spain, the Czech Republic, Denmark, Lithuania, Austria, the Netherlands, etc.

The great interest in several crops as sources for obtaining biofuels is the continuous reduction of fossil raw materials, the maximum of which will occur by 2030. That is why the production of biofuels as an alternative energy source is particularly relevant at present.

2. PURPOSE AND OBJECTIVES

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

The following tasks are specified to achieve the set goal:

- To investigate the effect of different nitrogen fertilization rates and harvesting stages on the growth and development of triticale grown for green mass.
- To study the effect of different nitrogen fertilization rates and harvesting stages on physiological indicators of triticale grown for green mass.
- To study the effect of different nitrogen fertilization rates and harvesting stages on the green biomass's structural elements.
- To study the effect of nitrogen fertilization rates and harvesting stages on green biomass yield.
- To study the effect of different rates of nitrogen fertilization and harvesting stages on the quality of the production for biofuel.

3. MATERIAL AND METHODS

3.1. Experimental methodology

The study was carried out at the experimental field of Agricultural University - Plovdiv. The experiment was carried out using the method of fractional plots in 4 replications, with the 20 m^2 size of the plots.

The following factors were studied:

Factor A - triticale varieties:
A1 - variety Musala
A2 - variety Atila

Factor C - harvesting stages:
C1 - heading
C2 – milky ripe

Factor B - fertilization rates:
B1 – N 0
B2 – N 12
B3 – N 16
B4 – N 20
B5 – N 24

Factor D - Conditions of the year
D1 – 2013/2014
D2 – 2014/2015
D3 – 2015/2016

3.2. Description of the varieties:

• Musala variety - Selected in a seed house-Sadovo from Prof. Dr. Iliya Stankov. Medium early variety, rye type. It exceeds the yield of barley, rye, and wheat variety Sadovo 1. It has completely resistant to powdery mildew, brown and black rust and is tolerant to fusarium. It is suitable for cultivation in all regions of the country, including on acidic and saline soils, with direction for grain and green mass in mixed crops with leguminous crops (vetch and peas). The variety is hardy, tolerant to the main diseases, has a fast rate of development, and early ripening, with excellent winter resistance. It grows well on all soil types, including acidic and saline ones. It responds well to mineral fertilization and gives a good yield in "economy mode." Suitable for use in several directions - the grain as a concentrated fodder, for addition to flour up to 30%, for the production of ethanol or diesel, fresh mass for ensiling in milk-wax maturity (especially in combination with leguminous crops in a mixed crop) or mowed more when flowering.

• Atila variety. Created at DZI General Toshevo. The variety is medium injured. The stem is 130-142 cm high. Resistant to lodging, winters successfully at shallow temperatures, in windy places without snow cover. Highly drought tolerant and resistant to powdery mildew, brown, yellow, and black rust, fusarium, and septoria. It does not require high rates of nitrogen fertilization. It is grown successfully on weak and acidic soils. The spikelets are dense, with high density and good resistance to plowing. The variety has a high productive potential of 10 - 11 t/ha green mass and over 700 kg/da grain. The mass of 1000 grains is between 48 and 52 g, and the hectoliter weight is 68-70 kg. The variety is characterized by a very good above-ground biomass, which makes it suitable not only for grain but also for silage production, biogas, and bioethanol.

3.3. Agrotechnics of experience

The experiment is based on the predecessor canola. Immediately after harvesting the predecessor, the area was disked with subsequent plowing to a depth of 20-25 cm to break up plant residues. With the primary tillage, 15 kg/da P_2O_5 and 10 kg/da K_2O were applied.

Pre-sowing treatment includes harrowing cultivation. Nitrogen fertilization was done with ammonium nitrate (NH4 NO3), with 1/3 of the nitrogen applied pre-sowing and 2/3 in early spring. The amounts of nitrogen fertilizer introduced were determined based on the tested fertilization levels and are as follows: 0, 12, 16, 20, and 24 kg a.v/day. The sowing of triticale was carried out with a plot seeder - on October 22, 2013, October 16, 2014, and October 20, 2015, with a row spacing of 12-15 cm and a seeding density of 450 k. s./m^2, at a depth of 4-6 cm, after which the area is leveled.

Weed control was not carried out during the three years.

In the second and third years of the experiment, the crop was attacked by wheat leech and brown rust. The treatment of the plants against the wheat leech was carried out twice (in the boot stage and heading stage s) with the insecticide Decis 2.5 EC - 40 ml/day, and against the brown rust, with the fungicide Allegro SK - 100 ml/day.

The green mass of triticale is harvested in two stages - mass heading and milky ripe.

4. SOIL AND AGROCLIMATIC CHARACTERISTICS

Regarding the agroclimatic characteristics during the experiment, the main influence is the meteorological factors (air temperature and precipitation), their combination and distribution during the vegetation, determining the crop's growth, development, and productivity during the years of cultivation.

The analysis of these factors shows specificity about average monthly temperatures and especially moisture (the amount and distribution of precipitation during the growing season), which is one of the main factors for obtaining high yields.

The data characterizing these factors during the three experimental years in the study area is indicated in Fig. 1 and 2.

The dates showed that in Central South Bulgaria, the values of the average daily temperatures, for the most part, exceed those of the perennial (fig. 1). Temperature values below those of the multi-year period are insignificant.

Significant deviations from the average monthly temperatures in October were not observed in all three years of the study. They range from 11.6^{0}C to 12.9^{0}C, with a multi-day average monthly temperature of -12.6^{0}C.

Average monthly temperatures in November exceed those of the multi-year period by 1.7 ^{0}C in the first year and by 3.9^{0}C in the third.

In the years of study, the same trend is preserved in the winter months (XII, I, II). The only exceptions are the months of December 2013 and January 2016, during which the recorded temperature was lower than that of the multi-year period. A short-term drop in the absolute minimum temperatures was registered in December 2013 (- 10.4^{o}C). , in January 2015 and 2016 (- 14.2 ^{o}C; - 17.5 ^{o}C), but due to snow cover and suspended vegetation, they do not negatively affect the wintering of plants.

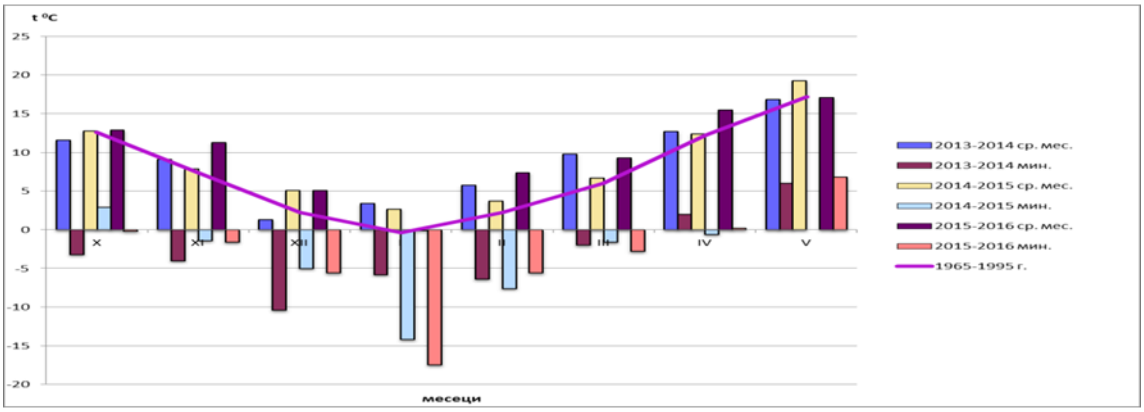

Figure 1 Average monthly and minimum temperatures during the years of study (°C)

During the years of the experiment, the average monthly temperatures recorded in March (9.8^{o}C; 6.7; 9.3^{o}C) vastly exceeded those of the multi-year period (6.0^{o}C). The most significant differences between the average monthly temperatures and those of the multi-year period were reported in February 2015 (5.2^{0}C) and March 2014 (3.8 ^{o}C).

Average monthly temperatures in April 2014 and 2015. have almost the same values, both among themselves (12.7 oC; 12.4 ^{o}C) and with the multi-year period (12.2^{o}C). The temperature was relatively higher in the third year of the study (15.5 ^{o}C).

The differences in average monthly temperatures in the first and third year of May (16.9 ^{o}C; 17.1 ^{o}C) and the multi-year period (17.2 ^{o}C) are insignificant. Again, this period recorded higher temperatures in May 2015 (19.3 ^{o}C).

The amount of precipitation in the experiment area (Central South Bulgaria) in all three years exceeded that of the multi-year period (321 mm) (Fig. 2).

The highest amount of precipitation was recorded in 2014/2015 (579.1 mm). The amount of rainfall was lower in 2013/2014 (395.5 mm) and the weakest in 2015/2016. (336.8mm).

Although the smaller amount of precipitation compared to the other two years, the third year is characterized by its relatively more even distribution during the vegetation and provision of sufficient moisture in the critical stages of the crop's development. Drought was observed only in December, when precipitation was only 3.6 mm.

The amount of precipitation that fell in the first year exceeds that of the third year by 242.3 mm, but unlike it, their distribution during the vegetation is uneven. The difference in precipitation in the second and third years is only 58.7 mm.

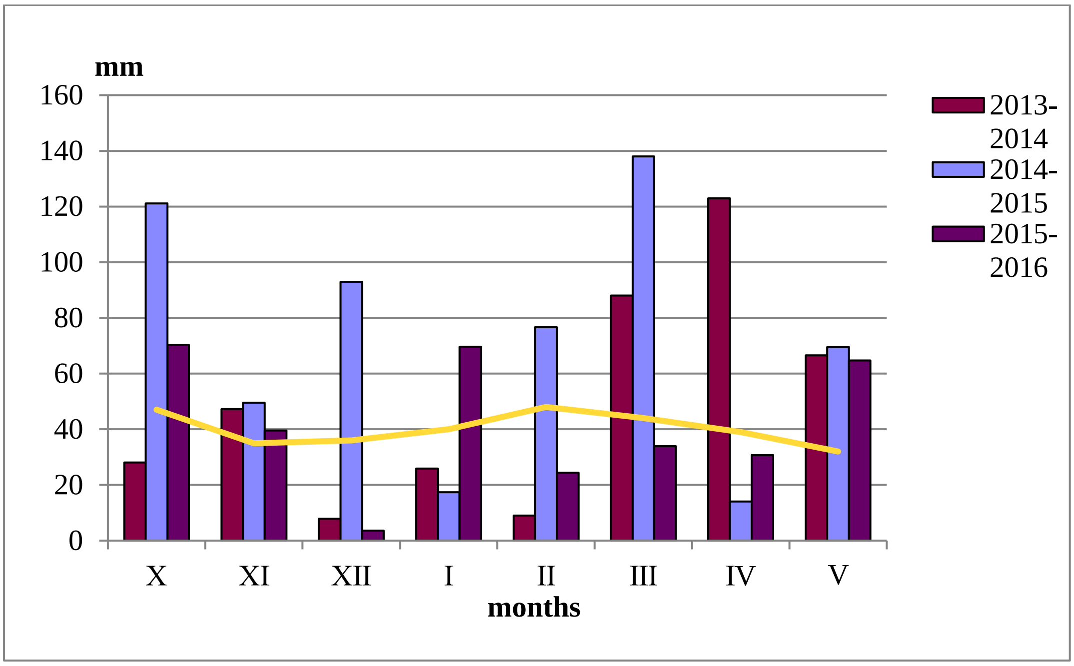

Figure 2 Amount of average monthly precipitation during the years of study (mm)

Severe droughts in the years of the experiment were observed in December and February of the first year (7.8 and 9.0 mm) and in January and April of the second (17.4 mm and 14.0 mm).

Characteristics of the first two years are the abundant rainfall recorded in October and March (121.1; 138.0 mm) of 2014/2015 and April of 2014 (123.0 mm).

During the years of the experiment, the highest amount of precipitation in October and November was recorded in the second year, 2014/2015 (170.6 mm), followed by the third year, 2015/2016 (109.9 mm).

Lower, compared to the other two years and the multi-year period (82 mm), is the precipitation in the fall of 2013 (75.3 mm).

The sum of precipitation in the winter months (December, January, February) of the second year of the study had higher values (187.0 mm) compared to the other two years (43.6; 97.6 mm) and the multi-year period (124mm).

The total amount of precipitation in the spring (March, April) of the first and second years of laying the experiment (211.0 mm; 152.0 mm) exceeded that of the third year by 146.4 mm; 87.4 mm, as well as in the multi-year period - with 127.0 mm; 69 mm.

In all three years of study, the precipitation that fell in May was almost evenly distributed (66.5 mm; 69.5 mm; 64.7 mm). It exceeded about twice the rain that fell in the multi-year period in that month (32mm).

5. RESULTS AND DISCUSSION

5.1. Effect of nitrogen fertilization and harvesting stages on phenological development of triticale for green mass

During all three years of the experiment, the stages of development in the region of Central South Bulgaria occurred at different times depending on the weather conditions and the specifics of the development of the tested varieties (Table 1).

Table 1 Phenological development of the studied triticale varieties

Stage s of development	Years					
	2013 – 2014		2014 – 2015		2015-2016	
	Varieties					
	Musalla	Atila	Musalla	Atila	Musalla	Atila
Sowing	22.10	22.10	16.10	16.10	20.10	20.10
Emergence (BBCH-11)	31.10	31.10	24.10	27.10	29.10	29.10
3rd leaf (BBCH -13)	15.11	15.11	07.11	12.11	10.11	13.11
Tillering (BBCH -21)	07.12.	07.12	05.12	10.12	01.12	07.12
Stop of vegetation	14.12	14.12	19.12	19.12	28.12	28.12
Vegetation renewal	23.02	23.02	20.02	20.02	22.02	22.02
Boot stage (BBCH- 43)	23.03	13.03	25.03	27.03	21.03	25.03
Heading (BBCH -57)	25.04	15.04	27.04	07.05	22.04	28.04
Vegetation period from germination to heading	176	166	185	192	176	182
Milky ripe (BBCH- 75)	15.05	29.04	11.05	21.05	09.05	16.05
Vegetation period from emergence to milky ripe	196	180	199	206	193	200

Significant differences in the development of triticale and, more significantly, between individual varieties were observed in the first year of the experiment compared to the other two years.

This year, until the onset of the boot stage, genotypic differences in the development of the varieties are not established, and their stages co-occur.

A discrepancy in the development of the two varieties is established only in the boot stage, when the climatic factors, temperature / 6.5 oC/ and humidity / 84.4 mm/, are closer to the requirements of the Atila variety.

As a result, the variety Atila entered the boot stage for 18 days (13.03. 2013).

The later increase in average daily temperatures during the second and third ten days of March (11.5°C; 10.7°C) and lower amounts of precipitation (27.3 mm; 10.6 mm) favor the development of the Musala variety. However, it cannot compensate and enters the boot stage 10 days later (23.03.) than the variety Atila.

The vegetation period from heading to milky ripe, depending on the years and varieties of cultivation, occurs after 14 to 20 days for the Musala variety and after 14-18 days for the Atila variety.

The vegetation period until the flowering stage varies from 176 to 192 days. The second and third years are longer in the Atila variety and the first year in the Musala variety.

The large amounts of precipitation that fell during boot stage-making in the Musala variety / 113.6 mm / extended the making and milky ripe by 7-16 days compared to the Atila variety, depending on the year of cultivation.

As a result, the growing season in 2013/2014 was 196 days long for the Musala variety and 180 days for the Atila variety. The growing season was longer in 2015/2016 – 193; 200 days, and the longest in 2014/2015 – 199; 206 days.

The necessary temperature sum for this period from germination to maturity in the Musala variety and Atila variety is, on average, 1353 °C; 1317°C, and from germination to milky ripe - 1615°C; 1569°C.

5.2. Influence of nitrogen fertilization and harvesting stages on the physiological parameters of triticale for green mass.

Leaf area is an indicator directly related to photosynthetic activity. The biological yield of the plant is formed (up to 93-98%) in the process of photosynthesis, in which the role of the leaves, as the main assimilating organs, is paramount. From the size of the leaf area, the speed of its formation, and the duration of its work under optimal conditions for the development of the crop, the size of the biological, respectively, the economic yield depends.

Table 2 presents the data on the area of the first flag leaf (cm2) and the length and width of the flag leaf (cm).

The table shows that the varieties studied show differences in the assimilation area.

Notably, the leaf area of the variety Atila is significantly greater than that of the variety Musala. The results shown in table 3 show that the harvesting stage of the green biomass of triticale and the different nitrogen fertilizer rates lead to significant changes in the parameters determining leaf gas exchange.

From the analyzes made for leaf gas exchange in the two triticale cultivars tested and the two harvesting stages, it can be seen that the reported rate of net photosynthesis in the Musala variety exceeds that in the Atila variety.

Using nitrogen fertilizers in the heading stage in both tested varieties positively affects the net photosynthesis rate. Therefore the values of this indicator are higher compared to the control variants.

In this stage, in the Musala variety, the most substantial positive effect on the net photosynthesis rate was observed at the N20 fertilization rate, where a value of 21.98 μmol m-2 s-1 was recorded. While in the Atila variety, a positive but less pronounced effect on photosynthesis was recorded compared to the Musala variety.

As the vegetation progressed, both cultivars observed a significant decrease in net photosynthesis rate.

In the milky ripe stage of the Musala variety, the rate of photosynthesis in the fertilized variants is lower compared to the unfertilized variants. The only exception is the fertilizer version with N16 (12.54 mmol m-2 s-1), where a proven positive effect on the rate of photosynthesis was reported.

In contrast to the Musala variety, in the Atila variety, in all fertilized variants, the effect on the rate of photosynthesis is positive. Still, here, too, the highest values are in the variant fertilized with N16 (11.70 mmol m-2 s-1).

Regarding the intensity of transpiration, the data in Table 3 show that in both stages of harvesting, the power of transpiration was significantly increased due to the greater sensitivity of the Atila variety to drought. In the Musala variety, which was selected in this region and is better adapted to high temperatures, the values of this indicator are lower.

The oral conductivity of the tested cultivars in the heading stage varies within narrow limits from 0.04 to 0.08. In both test cultivars and retraction stages, stomatal conductance was lowest in the control variants. In nitrogen-treated variants, stomatal conductance differences were negligible.

Plastid pigments in triticale leaves are influenced by the variety and the stages of crop harvesting and mineral fertilization (Berova et al., 2007) (Table 4).

The data in the table shows that the content of plastid pigments was the lowest in the leaves of the control variants. The content of plastid pigments in both stages of the harvest was higher in the Musala variety than in the Atila variety.

The content of plastid pigments in the leaves (chlorophyll A, chlorophyll A+B and carotene) in the Musala variety increases with fertilizer rates up to N16, after which their content slightly decreases. An exception is the Xlorophyll (B) content, where the values continue to increase until the variant - N20.

In the Atila variety, the highest values of all plastid pigments were recorded at nitrogen fertilizer rate N20, after which their content slightly decreased at the highest doses.

Table 2 Leaf area of a flag leaf of central tillers in the stage of emergence and milky ripe

Varieties	Musala variety			Atila variety		
	Heading stage					
Fertilizer rates N/kg/da	Length on the flag leaf, cm	Width on the flag leaf, cm	Area of 1 flag leaf, cm^2	Length on the flag leaf, cm	Width on the flag leaf, cm	Area of 1 flag leaf, cm2
N_0	21,72^d	1,86 ^{d}	25,46^d	29,09d	2,04d	37,35d
N_{12}	23,50 ^{c}	2,03 ^{c}	30, 07 ^{c}	31,51b	2,11c	41,8c
N_{16}	26,09^a	1,90 ^{d}	31,22^b	31,95b	2,21a	44,49a
N_{20}	25,67^a	2,11^a	33,37^a	32,75a	2,20a	45,32a
N_{24}	23,29$_d$	2,00^c	29,31^c	31,95b	2,07d	39,60d
N_{12-24}	24,05	2,18	29,89	31,15	2,13	41,71
Milky ripe stage						
N_0	17,35d	1,66d	18,17d	21,09d	1,85d	24,55d
N_{12}	22,00b	1,98a	27,50b	25,43b	1,95c	31,26c
N_{16}	24,40a	1,88b	28,92a	26,24b	2,16a	35,76a
N_{20}	23,90a	1,96a	29,57a	27,09a	2,15a	36,81a
N_{24}	22,05b	1,99a	27,60b	26,27b	2,12a	35,08b
N_{12-24}	21,94	1,89	26,35	25,27	2,05	32,69

*Evidence of differences at $P < 0.05$ if they do not have the same letters

Table 3 Parameters of leaf gas exchange

Fertilizer rates N/kg/da	Rate of net photosynthesis (A) µmol m^{-2} s^{-1}		Интензивност на транспирацията (E) mmol m^{-2} s^{-1}		Устична проводимост (gs) mmol m^{-2} s^{-1}	
	Heading stage	Milky ripe stage	Heading stage	Milky ripe stage	Heading stage	Milky ripe stage
	Musala variety					
N_0	18,08c	10,65b	0,14d	1,21b	0,04d	0,05d
N_{12}	16,77d	6,86d	0,18c	1,03c	0,07a	0,06c
N_{16}	16,69d	12,54a	0,34b	1,34a	0,07a	0,07b
N_{20}	21,98a	8,67c	0,44a	0,90d	0,06b	0,08a
N_{24}	21,47b	9,40c	0,42a	1,21b	0,06b	0,07b
Atila variety						
N_0	16,66d	9,80d	0,97d	0,92d	0,04d	0,05d
N_{12}	17,26c	9,94d	1,05c	1,32c	0,06b	0,07a
N_{16}	16,49d	11,70a	1,12b	1,79a	0,07a	0,07a
N_{20}	19,85a	10,67c	1,18a	1,38c	0,06b	0,06b
N_{24}	19,02b	11,43b	1,01c	1,58b	0,06b	0,06b

**Evidence of differences at $P < 0.05$ if they do not have the same letters*

Table 4 Amount of plastid pigments (mg/g)

N/kg/da	chl (A)		chl (B)		chl (A+B)		Каротин	
	Musala variety							
	Graduation	Milky maturity	Graduation	Milky maturity	Graduation	Milky maturity	Graduation	Milky maturity
N_0	1,80d	1,31d	0,85d	0,48d	2,65d	1,79d	0,67d	0,61d
N_{12}	2.02c	1,54c	0,89c	0,55d	2,91d	2,09c	0,70d	0,65d
N_{16}	2,89a	2,36a	0,91c	0,83b	3,80a	3,19a	1,09a	0,90a
N_{20}	2,75a	2,28a	0,98a	0,87a	3,73a	3,15a	0,98b	0,89a
N_{24}	2,64b	2,26a	0,94b	0,86a	3,58b	3,12a	0,89c	0,88a

Atila variety								
N_0	1,48d	1,29d	0,64d	0,37d	2,12d	1,66d	0,65d	0,60d
N_{12}	1,54d	1,35d	0,70d	0,41d	2,24d	1,76d	0,68d	0,63d
N_{16}	1,66d	1,48 d	0,76c	0,42d	2,42c	1,90d	0,92b	0,65c
N_{20}	2,30a	2,00a	1,02 a	0,70a	3,32a	2,70a	0,99a	0,82a
N_{24}	1,63d	1,41d	0,74d	0,48c	2,37c	1,89d	0,63d	0,59d

*Evidence of differences at $P < 0.05$ if they do not have the same letters

5.3. Productivity of triticale for green mass

5.3.1. Effect of nitrogen fertilization and harvesting stages on green mass yield of triticale, /kg/da.

The main criterion determining the economic qualities of a given crop and variety is its productivity. Crop harvesting stages and nitrogen fertilization are essential factors affecting it.

From the data in Table 5, it can be seen that in both harvesting stages (heading and milky ripe), the highest yield of green biomass of the Musala cultivar was obtained in the third year of the experiment. Although the lower amounts of precipitation (336.8mm) and higher temperatures compared to the other two years, 2016 is characterized by their relatively more even distribution during the growing season and provision of sufficient moisture in the critical stages of the variety's development, which favors the better development of the Musala variety, selected in the drier and warmer region of southern Bulgaria.

In the third year of 2016, the highest yield of green biomass was realized, which in the heading stage, depending on the nitrogen fertilizer norms, varies from 3740 kg/day to 4746 kg/day.

Depending on the stage, yields obtained in the second year of the study were inferior to those of the third, with an average of 830 kg/da; 290 kg/day.

The lowest green mass yields of the Musala variety were obtained in the first experimental year. They range from 2042 kg/da to 3020 kg/da.

Table 5 The yield of green mass in the heading stage and milky ripe in the Musala variety

Fertilizer rates N/kg/da	Heading stage					Milky ripe stage				
	Years			Average	%	Years			Average	%
	2014	2015	2016			2014	2015	2016		
N_0	2042^{c}	2758^{e}	3740e	2847	**100,0**	3500^{d}	3730^{e}	3920e	3717	100,0
N_{12}	2672^{b}	3301^{d}	4092d	3355	**117,8**	3576^{c}	4378^{c}	4606c	4187	**112,6**
N_{16}	2688^{b}	3771^{b}	4528b	3662	**128,6**	3826^{b}	4458^{b}	4778b	4354	**117,1**
N_{20}	3020^{a}	3815^{a}	4746a	3860	**135,6**	3884^{a}	4630^{a}	5130a	4548	**122,4**
N_{24}	2680^{b}	3633^{c}	4322c	3545	**124,5**	3823^{b}	4295^{d}	4506d	4208	**113,2**
Average	2620	3456	4286	3454	**121,3**	3722	4298	4588	4203	**113,1**

**Evidence of differences at $P < 0.05$ if they do not have the same letters*

In contrast to the Musala variety, the yield of green mass in the Atila variety followed exactly the opposite trend during the years of study (Table 6).

The years of the experiment characterized by lower amounts of precipitation and higher temperatures, which favor the development of the Musala variety, are less suitable for developing the Atila variety. Therefore, with this variety, in both harvesting stages, the highest yield of green mass was obtained in the first year of the experiment, and the lowest yield was recorded in the third year of the study.

In the first experimental year, green mass yields in the heading stage obtained from the Atila variety (from 2928 kg/da to 3915 kg/da) exceeded those of the Musala variety (from 2042 kg/da to 3020 kg/da).

Table 6 The yield of green mass in the heading stage and milky ripe of the Atila variety

Fertilizer rates N/kg/da	Heading stage					Milky ripe stage				
	Years			Average	%	Years			Average	%
	2014	2015	2016			2014	2015	2016		
N_0	2928d	2478e	2310e	2572	**100**	3811[e]	3368[e]	3198e	3459	**100**
N_{12}	3434c	3185d	3017c	3212	**124.9**	4104[d]	4071[d]	3991b	4055	**117,2**
N_{16}	3704b	3555b	3425a	3561	**138,4**	4342[b]	4160[b]	4056b	4186	**121,0**
N_{20}	3915a	3676a	3513a	3701	**143,8**	4698[a]	4572[a]	4200a	4490	**129,8**
N_{24}	3690b	3540c	3402a	3544	**137,8**	4222[c]	4099[c]	4010b	4110	**118,8**
Average	3534	3287	3133	3318	**129,0**	4235	4054	3891	4060	**117,4**

**Evidence of differences at P < 0.05 if they do not have the same letters*

In the milky ripe stage, on average, all fertilized variants exceeded the non-fertilized variants by 17.2 to 29.8% in yield.

The additional average yield obtained from applying the lowest N12 fertilizer rate in the milky ripe stage was 596 kg/da (17.2%).

Again, the N20-fertilized variant stands out with the highest yield, which on average over the three experimental years, exceeds the non-fertilized variant with an additional yield of 1031 kg/da (29.8%), with the difference of the extra yield between the lowest and the highest fertilizer rate is 435 kg/day.

The lowest yields in both varieties and harvest stages were obtained in the non-fertilized variants and the highest in the N20-fertilized variant.

From the statistical analysis of the degree of influence of the factors on the yield of green mass (Table 7), it is found that the harvesting stage - 82% - is the most significantly influenced, followed by fertilization and the year, respectively, with 79% and 54%. Of the factors considered independently, the influence of the variety is only 12%. In the interaction of the factors, it is evident that a significant impact is exerted by the interaction between variety*year – 78%, followed by variety*year*stage – 23%, and in third place year*stage – 14%. The influence of the other interactions of the factors is lower than that of those mentioned so far and varies between 2% and 11%.

Table 7 Strength of influence of the factors on the variation of fresh mass yield

factors	SS	DF	MS	F	P	%
variety	9,545771	1	9,545771	23,57	0,000003	12
year	8,530171	2	4,265085	105,29	0,000000	**54**
phenostage	3,394979	1	3,394979	838,10	0,000000	**82**
fertilization	2,820106	4	7,050265	174,05	0,000000	**79**
variety*year	2,600724	2	1,300362	321,01	0,000000	**78**
sort*stage	5,723267	1	5,723267	0,14	0,707447	2
year*stage	1,217411	2	6,087055	15,03	0,000001	14
variety*fertilization	3,636959	4	9,092397	2,24	0,066029	5
year*fertilization	8,568364	8	1,071046	2,64	0,009165	11
stage *fertilization	6,639110	4	1,659777	4,10	0,003341	8
variety*year*stage	2,136249	2	1,068125	26,37	0,000000	23
variety*year*fertilization	2,005264	8	2,506579	0,62	0,761388	3
variety*stage *fertilization	1,487802	4	3,719505	0,92	0,454559	2
year*stage *fertilization	6,042726	8	7,553407	1,86	0,068120	8
variety*year*stage *fertilization	1,849649	8	2,312061	0,57	0,800989	2

SS – the sum of squares, DF – degrees of freedom, MS – square of the mean value of the squares, F – the ratio between quantities, p – degree of evidence of the factor, % – degree of influence of the factor

These results indicate that triticale has excellent potential for inclusion in sustainable energy crop rotations for biomass production under less favorable growing conditions.

5. 3. 2. Influence of nitrogen fertilization and harvesting stages on the total and productive tillering in triticale

Sowing density is determined by the number of sprouted, overwintered, and harvested plants. It depends on the climatic factors during the years of research, the tested varieties, and the applied agro-technique of cultivation. Twining is an essential means of self-regulation of crop density. The density of sowing is directly related to the economic yield when harvesting the crop.

The data in Tables 8 and 9 show that depending on the weather conditions, tested varieties, harvesting stages, and nitrogen fertilizer rates, the number of tillers/m2 varies widely.

During the years of research, significant varietal differences were observed in the sowing density, which later affected the magnitude of the yield.

In the heading stage of the Musala variety, the highest sowing density was recorded in 2016 and the lowest in 2014, which correlates with the green biomass yields obtained.

The same trend regarding the tillering of plants by years and varieties is also observed in the milky ripe stage, but with slightly higher values.

In this stage, the number of plants in 2016 varies from 773 to 987 pcs./m2, and in the milky ripe stage, from 779 pcs./m2 to 993 pcs./m2. Plant density was slightly lower in 2015, from 665 pcs./m2 to 905 pcs./m2 in the heading stage and from 670 pcs./m2 to 912 pcs./m2 in the milky ripe stage. The sowing density was the lowest in 2014 when the yields obtained from the Musala variety were also the lowest.

Regarding the density of sowing, the opposite trend is observed in the Atila variety. 2014 stands out with the highest total tillering in the heading stage, during which the number of plants varied from 681 pcs./m2 to 908 pcs./m2, and with the lowest, 2016, when they were listed from 587 pcs. ./m2 to 737 pcs./m2, and in the milky ripe stage, respectively from 689 pcs./m2 to 915 pcs./m2; from 594 pcs./m2 to 748 pcs./m2, which also corresponds to the obtained green biomass yields.

On average, for the years of the experiment, depending on the harvesting stage, the percentage of productive twinning in the Musala variety exceeded that of the Atila variety by 1.04% and 1.57%.

In the Atila variety, in both harvest stages, compared to the non-fertilized version, the productivity ratio at the first fertilization rate increased to 61.14; 62.46%, on the second, N16, to 63.13; 64.22, on the third, N20 to 64.68; 68.87%. At the highest fertilizer rate (N24), the percentage of productive tillers relative to the control depending on the harvest stage increased the most by 4.15%, 4.98% for the Musala variety, and 4.64%; 5.98% in the Atila variety.

The percentage of productive tillers is the highest in the variant, with 24 kg/da in the Musala variety - 65.69%; 68.62%. And for Atila variety - 64.80%; 67.19%. The percentage compared to the control increased by 4.15%, 4.98% for the Musala variety, and 4.64%; 5.98% for the Atila variety.

From the calculations made in this way of the whole tillering, the number of spikelets-bearing stems, and the productive tillering, it is established that:

A solid varietal response was observed in the indicators of general tillering, some spikelets-bearing stems, and productive tillering.

The Musala variety formed the highest values of the indicators in 2016, and the Atila variety in 2014.

On average, for the experiment period, more tillers/ m2, spikelets/ m2, and productive tillers formed the Musala variety.

Fertilization with nitrogen increases the number of spikelets-bearing stems formed per unit area in both stages by 12.7%; 13.4% at the lowest level of fertilization (N12) to 46.8%; 43.1% at the highest level (N24) of fertilization, as well as the percentage of productive tillers.

Table 8 Total shoots of plants in the heading stage, number of shoots/m2

variety	N/kg/da	Number of tillers of / m^2			Average
		2014 г.	2015г.	2016г.	
Musalla	N_0	491	665	773	643
	N_{12}	563	751	845	719
	N_{16}	634	842	924	800
	N_{20}	695	904	985	861
	N_{24}	697	905	987	863
Atila	N_0	681	607	587	625
	N_{12}	703	673	631	669
	N_{16}	785	712	684	727
	N_{20}	903	815	735	818
	N_{24}	908	817	737	820

Table 9 Total tillering in the milky ripe stage, number of tillers

variety	N/kg/da	Number of tillers of / m^2			Average
		2014 г.	2015г.	2016г.	
Musalla	N_0	496	670	779	648
	N_{12}	568	756	852	725
	N_{16}	641	848	931	807
	N_{20}	703	911	992	869
	N_{24}	708	917	998	874
Atila	N_0	689	617	594	633
	N_{12}	712	683	639	678
	N_{16}	793	754	695	747
	N_{20}	911	825	746	827
	N_{24}	915	827	748	830

Table 10 Number of spikelets-bearing stems/m2 and productive fertility, % in the heading stage

variety	N/kg/da	Number of spikelets-bearing stems /m^2				Productive tillers %			
		2014г.	2015г.	2016г.	Average	2014г.	2015г.	2016г.	Average
Musalla	N_0	292	411	489	397	59,47	61,80	63,36	61,54
	N_{12}	341	468	542	450	60,56	62,31	64,14	62,33
	N_{16}	397	543	603	514	62,61	64,48	65,26	64,11
	N_{20}	446	592	657	565	64,17	65,49	66,70	65,45
	N_{24}	450	595	660	568	64,45	65,75	66,87	65,69
	Average	**385**	**522**	**590**	**499**	**62,25**	**63,97**	**67,27**	**63,82**
Atila	N_0	418	365	348	377	61,08	60,13	59,28	60,16
	N_{12}	437	412	379	409	62,16	61,21	60,06	61,14
	N_{16}	503	450	423	458	64,07	63,20	62,13	63,13

	N_{20}	587	528	465	527	66,00	64,78	63,26	64,68
	N_{24}	601	530	467	533	66,18	64,87	63,36	64,80
	Average	**509**	**457**	**416**	**460**	**63,89**	**62,84**	**61,62**	**62,78**

Table 11 Number of spikelets-bearing stems/m2 and productive fertility, % in the milky ripe stage

variety	N/kg/da	Number of spikelets-bearing stems /m^2				Productive tillers %			
		2014г.	2015г.	2016г.	Average	2014г.	2015г.	2016г.	Average
Musalla	N_0	305	420	505	410	61,49	62,68	64,82	62,99
	N_{12}	353	480	555	462	62,15	63,49	65,14	63,59
	N_{16}	415	553	637	535	64,74	65,21	68,42	66.12
	N_{20}	477	622	688	595	67,85	68,27	69,35	68,49
	N_{24}	482	627	693	602	68,07	68,37	69,43	68,62
	Average	**406**	**540**	**616**	**521**	**64,86**	**65,60**	**67,43**	**65,96**
Atila	N_0	428	378	358	388	62,12	61,26	60,26	61,21
	N_{12}	449	428	394	424	63,06	62,66	61,65	62,46
	N_{16}	519	486	438	481	65,19	64,45	63,02	64,22
	N_{20}	627	551	485	553	68,83	66,78	65,01	66,87
	N_{24}	632	554	490	559	69,07	66,98	65,51	67,19
	Average	**531**	**479**	**433**	**481**	**65,65**	**64,43**	**63,09**	**64,39**

*5. 3. 3. Influence of nitrogen fertilization and harvesting stages on the biometric indicators of the cro*p

- **Plant height, (cm).**

The indicators influencing green biomass yield are the plants' height and the structural elements forming the yield.

Although plant height is a varietal trait, Table 12 shows that its values vary depending on nitrogen fertilization rates, harvest stages, and growing conditions.

Comparing the three experimental years, it is found that the more significant amount of precipitation in the boot stages and heading in 2014 and 2015 in both varieties creates a prerequisite for forming plants with taller stems. The plants formed the highest stems in the second year, 2015, followed by 2014.

The drier and warmer climate in 2016 caused these stages to occur more rapidly, resulting in the plants forming slightly shorter stems in the test specimens.

Table 12 Plant height

N kg/da	Heading stage				Milky ripe stage			
	Years							
	2014	2015	2016	Average	2014	2015	2016	Average
Musala variety								
N_0	108,6d	111,0^{d}	105,0^{d}	108,2	118,9 ^{d}	121,0 ^{d}	116,3 ^{d}	118,7
N_{12}	116,2 ^{c}	120,1^{c}	114,0^{c}	116,8	129,1 ^{c}	132,0 ^{c}	127,4 ^{c}	129,5

N_{16}	$119,3^{b}$	$123,0^{b}$	$117,0^{b}$	119,8	$132,0^{b}$	$136,0^{b}$	$129,6^{b}$	132,5
N_{20}	$125,0^{a}$	$128,2^{a}$	$123,4^{a}$	125,5	$134,0^{a}$	$141,2^{a}$	$130,7^{a}$	135,3
N_{24}	$124,1^{a}$	$127,4^{a}$	$123,0^{a}$	124,8	$132,9^{a}$	$140,5^{a}$	$130,0^{a}$	134,5
Atila variety								
N_{0}	118,7d	$119,5^{d}$	$116,4^{d}$	118,2	$124,1^{d}$	$126,0^{d}$	$122,9^{d}$	124,3
N_{12}	$127,0^{c}$	$129,4^{c}$	$125,2^{c}$	127,2	$136,2^{c}$	$138,0^{c}$	$134,5^{c}$	136,2
N_{16}	$130,0^{b}$	$132,8^{b}$	$129,3^{b}$	130,9	$141,5^{b}$	$145,6^{b}$	$139,2^{b}$	142,1
N_{20}	$138,6^{a}$	$140,4^{a}$	$135,6^{a}$	138,2	$147,7^{a}$	$148,0^{a}$	$145,1^{a}$	146,9
N_{24}	$138,0^{b}$	$140,0^{a}$	$135,0^{a}$	137,7	$147,0^{a}$	$147,8^{a}$	$144,6^{a}$	146,5

*Evidence of differences at $P < 0.05$ if they do not have the same letters

• Stem thickness (cm).

The data in table 13 show that, depending on the specificity of weather conditions, nitrogen fertilizer rates, and harvesting stages, the indicators' values and the stems' thickness change during the years of cultivation.

In the first and third years of the experiment, the differences in the thickness of the stems in the heading stage were insignificant.

The growth of the plants at a higher height in 2015 is a prerequisite for the formation of plants with slightly thinner stems, from 4.8 mm in the unfertilized variants to 4.5 mm in the variant with the highest fertilizer rate in the Musala variety and from 5.0 mm to 4.7 mm in Atila variety.

In both harvesting stages averaged over the study period, the differences in stem thickness between the two cultivars were insignificant, with a slight predominance for cultivar Atila. This is due to the more special arrangement of its stem, which has a conical shape and is thicker at the base and thinner towards the tip. This makes it more resistant to lying down, despite its greater height. The thickness of the stems in the variety Atila varies from 4.9 mm to 5.2 mm in the heading stage and 5.1 mm to 5.4 mm in the milky ripe stage.

The stem of the Musala variety is thinner and cylindrical; its thickness varies from 4.7 mm to 0.5.1 mm in the heading stage and 4.9 mm to 5.3 mm in the milk stage.

Statistically proven differences in this indicator are observed between fertilization options within the variety.

Table 13 Stem thickness (mm)

Fertilizer rates kg/da	Stem thickness (mm)							
	Heading stage				Milky ripe stage			
	2014	2015	2016	Average	2014	2015	2016	Average
	Musala variety							
N_{0}	5,2a	4,8a	5,3a	5,1	5,3a	5,1a	5,4a	5,3
N_{12}	5,1a	4,7b	5,2b	5,0	5,2b	5,0b	5,3b	5,2
N_{16}	5,0b	4,6c	5,2b	4,9	5,2b	5,0b	5,3b	5,2
N_{20}	4,9b	4,6c	5,1c	4,8	5,1c	4,9c	5,2c	5,0
N_{24}	4,8c	4,5d	5,0d	4,7	5,0c	4,8d	5,1d	4,9
	Atila variety							
N_{0}	5,3a	5,0a	5,4a	5,2	5,4a	5,3a	5,5a	5,4
N_{12}	5,2b	4,9b	5,3b	5,1	5,3b	5,2b	5,5a	5,3
N_{16}	5,2b	4,9b	5,3b	5,1	5,3b	5,1b	5,4b	5,3
N_{20}	5,1c	4,8c	5,2c	5,0	5,2c	5,1c	5,3c	5,2
N_{24}	5,0d	4,7d	5,1d	4,9	5,1d	5,0d	5,2d	5,1

**Evidence of differences at $P < 0.05$ if they do not have the same letters*

• Number of leaves per plant

In cereal crops, the leaf mass is finally formed in the leveling stage; therefore, some leaves begin to dry in the milky ripe stage, and the leaf mass decreases or remains unchanged.

More significant differences regarding the number of leaves were observed between the years of study and nitrogen fertilizer rates, while there were almost none between the two varieties tested. (Table 14).

The weather conditions were better in the spring of 2015. The boot and leveling stages favor the formation of the most significant number of leaves in the cultivated varieties (from 12.17 to 15.21 in the Musala variety and from 12.28 to 15.44 pcs. for the Atila variety).

The drier and warmer climate during the same period of 2016 was the reason for the formation of a smaller number of leaves (from 10.61 to 13.33 pcs. in the variety Musala and from 10.81 to 13.45 pcs. in the variety Atila).

Plant height is directly proportional to the number of leaves, and plants with taller stems produce more.

Therefore, over the years and on average for the study period, the number of leaves in the Atila variety is slightly higher.

Table 14 Number of leaves per plant

Fertilizer rates kg/da	Number of leaves per plant							
	Heading stage				Milky ripe stage			
	2014	2015	2016	Average	2014	2015	2016	Average
	Musala variety							
N_0	11,40[d]	12,17[d]	10,61[d]	11,39	11,34[d]	12,00[d]	10,55[d]	11,30
N_{12}	12,70[c]	13,31[c]	11,52[d]	12.51	12,50[c]	13,27[c]	11,52[d]	12.43
N_{16}	13,28[b]	14,15[b]	12,18[b]	13,38	13,23[b]	14,11[b]	12,00[c]	13.11
N_{20}	14,10[a]	15,21[a]	13,33[a]	14,21	14,00[a]	15,10[a]	13,25[a]	14,12
N_{24}	14,00[a]	15,15[a]	13,25[a]	14,17	13,91[a]	15,00[a]	13,20[a]	14,03
	Atila variety							
N_0	11.52[d]	12,28[d]	10,81[d]	11,54	11.41[d]	12,15[d]	10,70[d]	11,42
N_{12}	12,81[c]	13,42[d]	11,63[d]	12,62	12,63[c]	13,35[c]	11,49[c]	12,49
N_{16}	13,44[b]	14,38[b]	12,55[b]	13,52	13,32[b]	14,10[b]	12,22[b]	13,21
N_{20}	14,25[a]	15,44[a]	13,45[a]	14,38	14,15[a]	15,25[a]	13,36[a]	14,25
N_{24}	14,20[a]	15,35[a]	13,37[a]	14,30	14,13[a]	15,35[a]	13,28[a]	14,12

**Evidence of differences at P < 0.05 if they do not have the same letters*

• Number of spikelets per plant

More significant differences in the number of spikelets are observed between the years of the study (Table 15).

The plants in 2014, from 1.3 to 1.8 pcs., and the largest in 2016, from 1.9 to 2.9 pcs. The exact opposite trend is observed in the Atila variety. A higher number of plants than the Musala cultivar was reported in the first year and a lower number in the remaining two years. Averaged over the three years, the differences between varieties are insignificant. On average, a slightly higher number of ears were recorded during the milky ripe stage, from 1.7 to 2.7 pcs. , for the Musala variety and from 1.6 to 2.6 pcs. in the Atila variety. The plants with the unfertilized variants formed the smallest number of spikelets, on average, for the period - 1.7 pcs. for the Musala variety and 1.6 pcs. in the Atila variety.

Table 15 Number of spikelets per plant

Fertilizer rates kg/da	Number of spikelets per plant							
	Heading stage				Milky ripe stage			
	2014	2015	2016	Average	2014	2015	2016	Average
	Musala variety							
N_0	1,3[d]	1,7[d]	1,9[d]	1,6	1,5[d]	1,7[d]	2,0[d]	1,7
N_{12}	1,5[c]	1,9[c]	2,0[c]	1,8	1,7[c]	2,0[c]	2,3[c]	2,0
N_{16}	1,6[c]	2,0[c]	2,2[c]	1,9	1,8[b]	2,3[b]	2,5[c]	2,2
N_{20}	1,8[a]	2,7[a]	2,9[a]	2,5	2,0[a]	2,9[a]	3,1[a]	2,7
N_{24}	1,8[a]	2,7[a]	2,9[a]	2,5	2,1[a]	2,9[a]	3,0[a]	2,7
	Atila variety							
N_0	1,7[d]	1,5[d]	1,4[d]	1,5	1,8[d]	1,6[d]	1,5[d]	1,6
N_{12}	1,8[c]	1,7[c]	1,6[c]	1,7	2,0[c]	1,8[c]	1,9[c]	1,9
N_{16}	2,0[b]	1,8[b]	1,7[c]	1,8	2,4[b]	2,0[b]	1,9[c]	2,1
N_{20}	2,8[a]	2,5[a]	2,0[a]	2,4	2,9[a]	2,3[a]	2,6[a]	2,6
N_{24}	2,8[a]	2,5[a]	2,0[a]	2,4	2,9[a]	2,3[a]	2,6[a]	2,6

In both varieties, with an increase in fertilizer rates, the number of clusters slightly increases up to the nitrogen fertilizer rate of 20 kg/da. In the stage of heading and milky ripe in the Musala variety, the number of spikelets on average for the period is 2.5 pieces; 2.7 pcs., and for the Atila variety, 2.4 pcs.; 2.6 pcs. The exact number of spikelets is formed at the highest fertilizer rate, 24 kg/da.

• Spikeletss length, (cm).

Even though the length of the ear is a genetically determined quantity, to a certain extent, it is influenced by climatic conditions and applied agricultural techniques (Table 16).

Moreover, slightly longer spikelets formed the variety Atila during the three years of study. For the study period, this indicator varies from 12.60 to 13.90 pcs. Moreover, from 11.68 to 13.76 pcs. for the Musala variety.

The lowest length values were recorded for the unfertilized variants in both varieties and stages. In the heading stage, 11.68 cm was recorded for the Musala variety and 12.60 cm for the Atila variety.

The inclusion of fertilization has a positive effect on spikelets' length.

Table 16 Spikelets length (cm)

Fertilizer rates kg/da	Spikelets length (cm)							
	Heading stage				Milky ripe stage			
	2014	2015	2016	Average	2014	2015	2016	Average
	Musala variety							
N_0	13,00[d]	11,17[d]	10,87[d]	11,68	13,24[d]	11,45[d]	10,84[d]	11,84
N_{12}	13,12[c]	11,78[d]	11,00[d]	11,96	13,86[c]	12,17[d]	11,21[d]	12,40
N_{16}	13,20[c]	12,41[c]	11,85[c]	12,62	13,88[b]	12,95[c]	12,05[c]	12,92
N_{20}	14,11[a]	13,82[a]	13,10[a]	13,76	14,28[a]	13,98[a]	13,76[a]	14,01
N_{24}	14,00[a]	13,80[a]	13,05[a]	13,61	14,25[a]	13,45[b]	12,00[c]	13,24
	Atila variety							
N_0	13,22[d]	12,45[d]	12,12[d]	12,60	13,50[d]	12,97[d]	12,62[d]	13,03
N_{12}	13,29[d]	12,58[d]	12,76[c]	12,88	14,60[c]	13,72[c]	13,39[c]	13,90

N_{16}	13,81[c]	13,64[b]	13,38[a]	13,61	15,10[b]	14,36[b]	13,80[a]	14,56
N_{20}	14,29[a]	13,90[a]	13,52[a]	13,90	15,72[a]	14,78[a]	13,91[a]	14,80
N_{24}	14,24[a]	13,76[a]	13,50[a]	13,81	15,23[a]	14,74[a]	13,83[a]	14,61

**Evidence of differences at P < 0.05 if they do not have the same letters*

5.3.4. Influence of nitrogen fertilization and harvesting stages on the morphological structure of green plants

The data on the mass of one plant for the cultivars grown in the two harvest stages of triticale are reflected in Table 17.

When analyzing the results in the table, it can be seen that in the first year of research and both harvesting stages, the plants of the Atila variety were heavier (from 12.52 g to 18.52 g; from 13.35 to 19.97 g). In the second and third years, variety Musala (from 16.00 g to 21.60 g; from 17.47 g to 24.31 g and from 18.48 to 25.38 g; from 22.02 to 28.69 g), which correlates with the amount of green mass yield.

On average, for the three experimental years and in both harvest stages, the plants of the Musala variety have a greater mass than those of the Atila variety.

The average plant weight of the cultivar Musala in the heading stage ranged from 15.67 g to 21.83 g and that of the cultivar Atila from 14.60 g to 19.92 g, with the plant weight of the cultivar Musala exceeding by 1.07 g to 2.12 g that of Atila variety.

After heading, the mass of the plants continues to grow, and in the milky ripe stage, in individual years and on average for the period, it increases significantly. In this stage, on average for the period, the mass of plants of the variety Musala (from 17.61 g to 24.32 g) exceeded that of the variety Atila (from 16.91 g to 22.85 g).

In both stages of harvesting, the mass of the plants is the lowest in the non-fertilized variants. Depending on the years of study, the mass of plants at the tillering stage of cultivar Musala varied from 12.52 g to 18.48 g, and at cultivar Atila, 11.50 g to 17.10 g. At the milky ripe stage, the mass ranged from 13.35 g to 22.02 g in the Musala variety and from 13.12 g to 20.65 g in the Atila variety. On average, for the period and in both harvesting stages, variety Musala slightly exceeds the plant mass of Atila.

With an increase in nitrogen fertilizer rates up to N20, a gradual increase in plant mass was observed in both varieties and harvest stages, after which, as a result of thinner stems, at the highest nitrogen rate, it slightly decreased, which also coincided with the obtained yields green biomass.

Table 17 Fresh mass of one plant (g)

Fertilizer rates kg/da	Heading stage				Milky ripe stage			
	Years							
	2014	2015	2016	Average	2014	2015	2016	Average
	Musala variety							
N_0	12,52[d]	16,00[d]	18,48[d]	**15,67**	13,35[d]	17,47[d]	22,02[d]	**17,61**
N_{12}	14,66[c]	18,51[c]	20,15[c]	**17,77**	15,27[c]	20,40[c]	24,81[c]	**20,16**
N_{16}	16,45[b]	20,91[a]	22,07[b]	**19,81**	18,07[b]	22,59[b]	26,98[b]	**22,55**
N_{20}	18,52[a]	21,60[a]	25,38[a]	**21,83**	19,97[a]	24,31[a]	28,69[a]	**24,32**
N_{24}	17,00[b]	19,20[b]	20,56[c]	**18,92**	17,54[b]	22,34[b]	26,03[c]	**21,97**
	Atila variety							
N_0	15,21[d]	11,50[d]	17,10[d]	**14,60**	16,96[d]	13,12[d]	20,65[d]	**16,91**
N_{12}	17,62[c]	12,50[d]	18,88[c]	**16,33**	18,63[c]	15,50[c]	22,77[c]	**18,96**
N_{16}	18,77[b]	14,83[b]	19,47[b]	**17,69**	19,95[b]	19,11[b]	23,91[c]	**20,99**
N_{20}	20,62[a]	17,17[a]	21,98[a]	**19,92**	22,25[a]	20,24[a]	26,05[a]	**22,85**

N24	18,10 c	15,35 c	19,73 c	**17,73**	19,66 b	18,19 b	24,85 b	**20,90**

**Evidence of differences at P < 0.05 if they do not have the same letters*

The data from the morphological analysis of the plants in the cultivated varieties and harvesting stages on average for the period of the experiment are reflected in Figures 3 and 4.

The largest total mass is the stems in tested varieties and harvesting stages. The plants of the Musala variety have slightly heavier stems - from 8.75 g to 12.48 g compared to the Atila variety, from 7.73 g to 10.81 g.

In second place are the leaves, but the differences between the cultivars are insignificant (in the variety Atila, it varies from 3.82 g to 5.51 g, and in the variety Musala, from 3.79 g to 5.46 g).

The mass of the spikelets is the lowest. However, even for them, the differences between the varieties are insignificant (for the Musala variety from 3.13% to 3.89%, and for the Atila variety from 3.05 g to 3.60 g).

In contrast to the heading stage, in the milky ripe stage, although slightly, the stems continue to grow and retain the highest mass concerning the total mass of the plant, but with higher values.

The weight of the leaves in the milky ripe stage decreases due to their full development in the leveling stage and the death of the lowest ones, and the weight of the spikelets increases significantly.

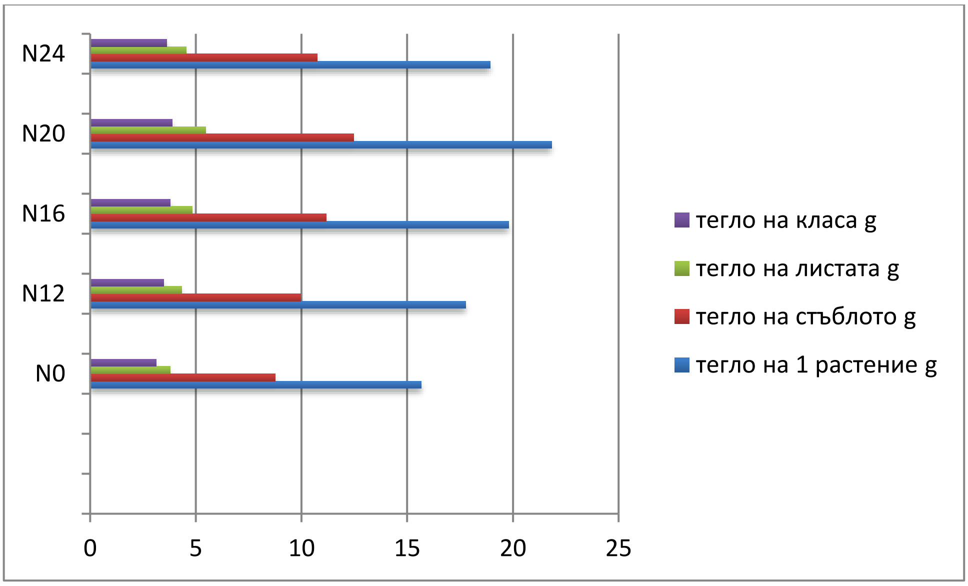

Figure 3 Morphological analysis of fresh plants, the average for the growing period in the heading stage, variety Musala, g

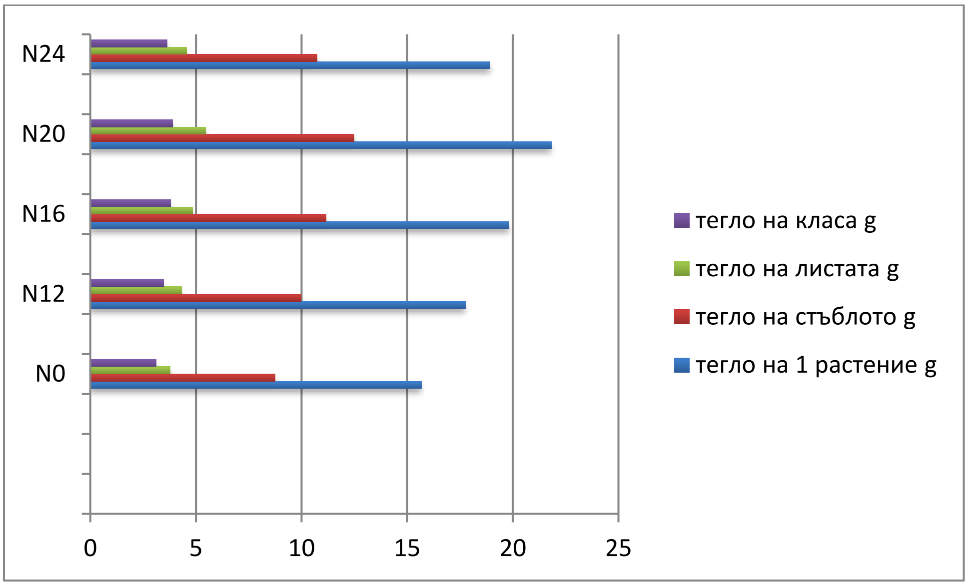

Figure 4 Morphological analysis of fresh plants, the average for the growing period in the heading stage, variety Atila, g

From the morphological structure of the plants made in this way, it is established that:

- ✓ The average biomass of a plant has higher values in the milky ripe stage. A higher mass was recorded for the Musala variety of the two varieties tested.
- ✓ Stems and leaves have the largest share in the formation of the yield of green mass in the heading stage and the milky ripe stage - stems and spikelets.
- ✓ Irrespective of the harvesting stage, the values of these indicators are the lowest in unfertilized variants in both varieties. With an increase in fertilizer rates, the values of all indicators increase up to the N20 fertilizer option.
- ✓ The percentage ratio of the organs of the plants in the flowering stage at different nitrogen fertilization rates follows the order: of stems: leaves: spikelets, and in the milky ripe stage : stems: spikelets: leaves.

5.4. Productivity of triticale, dry matter, and silage

5.4.1. Effect of nitrogen fertilization and harvesting stages on yield and dry matter content

The amount of biogas and methane obtained from one ton of silage depends on the dry matter content of the substrate. According to research by Amon (2006), plants' optimal dry matter content at harvest is 30 to 35 %. At this content, the most energy and biogas are obtained.

In addition, the dry matter content of whole triticale plants depends on the weather conditions during the growing years and the harvest stage.

The data in table 18 show the percentage of dry matter in the variety Musala in the heading stage and milky ripe. It can be seen from them that the highest percentage of dry matter, both in individual years and on average for the period, was recorded in 2016 (22.79%) (when the lowest amount of precipitation was recorded) and the lowest in 2015 (20.79%).

Table 18 Content of dry matter in the green mass of triticale in Musala variety, (%)

Retraction stages	Year	Dry matter, %					
		N_0	N_{12}	N_{16}	N_{20}	N_{24}	Average
Graduation	2014г.	21,85[c]	21,90[c]	21,91 [b]	22,00[a]	21,45[a]	21,82
	2015г.	20,85[b]	20,85[b]	20,87 [b]	20,97[a]	20,43[d]	20,79

	2016г.	22,82^{c}	22,84^{c}	22,90^{b}	23,30^{a}	22,11^{d}	22,79
	Average	**21,84**	**21,86**	**21,89**	**22,09**	**21,33**	**21,80**
Milky maturity	2014г.	32,47^{d}	32,51^{d}	32,63^{c}	32,97^{a}	32,81^{b}	32,68
	2015г.	30,48^{d}	30,50^{d}	30,56^{c}	30,84^{a}	30,47^{d}	30,54
	2016г.	33,21^{d}	33,41^{d}	33,64^{c}	33,98^{a}	33,81^{b}	33,61
	Average	**32,05**	**32,14**	**32,28**	**32,60**	**32,36**	**32,27**

**Evidence of differences at $P < 0.05$ if they do not have the same letters*

The dry matter values in 2015 were 1.03, 2.00 % lower than the other two years.

Of the variants tested, the lowest percentage of dry matter was recorded in the controls. Moreover, depending on the years of study, values range from 20.85% to 22.82%.

As the vegetation progresses, the content of dry matter in the raw material increases. The content of dry matter in both varieties tested in the stage of milky ripe is closer to the above-mentioned optimal values of dry matter (30-35%) for obtaining better quality silage, the use of which leads to obtaining more biogas. While in the heading stage, the average values of the dry matter are 21.80%, in the milky ripe stage, the content increases by 10.47%.

Table 19 Content of dry matter in the green mass of triticale in Atila variety, (%)

Retraction stage s	Year	Dry matter, %					
		N_0	N_{12}	N_{16}	N_{20}	N_{24}	Average
Graduation	2014г.	22,59^{d}	22,63^{d}	22,98^{c}	23,44^{a}	23,00^{c}	22,93
	2015г.	19,94^{c}	20,00^{b}	20,07^{b}	20,19^{a}	19,69^{d}	19,98
	2016г.	22,48^{d}	22,52^{d}	22,61^{c}	22,89^{a}	22,46^{d}	22,59
	Average	21,67	21,72	21,89	22,17	21,72	21,83
Milky maturity	2014г.	32,10^{d}	32,40^{c}	32,63^{b}	33,18^{a}	31,76^{d}	32,41
	2015г.	31,49^{d}	31,78^{c}	32,25^{b}	32,61^{a}	30,91^{d}	31,81
	2016г.	33,40^{d}	33,58^{d}	33,84^{c}	33,93^{a}	33,87^{b}	33,72
	Average	32,33	32,59	32,91	33,24	32,18	32,65

**Evidence of differences at $P < 0.05$ if they do not have the same letters*

Furthermore, in the Atila variety, the dry matter content in the milky ripe stage significantly exceeds the average value reported in the heading stage by 10.82% (table 19).

Musala and Atila varieties in this stage had the lowest dry matter values recorded in 2015 (31.81%).

As a result of the low amounts of precipitation in the period of milky ripe in 2016 (55.1mm) compared to the other two years, the highest values in the dry matter content were recorded (average for the period of 33.72%). The lowest values were recorded in the control variants and Atila variety.

With an increase in fertilizer rates up to the fertilizer option with 20 kg/da nitrogen, the dry matter content increases (average 33.24%), then decreases slightly (average 32.18%).

Dry matter yield is a function of green mass yield and dry matter content (tables 20 and 21).

Variety, nitrogen fertilization, and a complex of agrometeorological factors influence dry biomass.

In all tested variants over the years and on average over the study period, the lowest dry matter yield was obtained in the non-fertilized variants. The highest yields were obtained from the variant fertilized with 20 kg/da nitrogen, which exceeded the control over the years as follows: by 336 kg/day (27.5%), 430 kg/day (40.5%), 357 kg/day (33.4%); 375 kg/da (33.6%) additional yield.

Table 20 Dry mass yield of Musala variety kg/da

Fertilizer rates kg/da	Stages									
	Heading					Milky ripe				
	Year									
	2014	2015	2016	Average		2014	2015	2016	Average	
	кg/da	кg/da	кg/da	кg/da	% от N_0	кg/da	кg/da	кg/da	кg/da	% от N_0
N_0	446[d]	575 [d]	853 [d]	625	100	1136 [d]	1137 [d]	1302 [d]	1192	100
N_{12}	585[b]	688[c]	935 [d]	736	117,8	1163 [d]	1335[b]	1539[c]	1346	112.9
N_{16}	589[b]	787[a]	1037[b]	804	128,6	1248[b]	1362[b]	1607[b]	1406	117,9
N_{20}	664[a]	800[a]	1106[a]	857	137,1	1281[a]	1428[a]	1743[a]	1484	124,5
N_{24}	575[c]	742[b]	956[c]	758	121,3	1254[b]	1309[c]	1523[c]	1362	114,3

**Evidence of differences at $P < 0.05$ if they do not have the same letters*

Table 21 The yield of the dry mass of variety Atila, kg/da

Fertilizer rates kg/da	Stages									
	Heading					Milky ripe				
	Year									
	2014	2015	2016	Average		2014	2015	2016	Average	
	кg/da	кg/da	кg/da	кg/da	% от N_0	кg/da	кg/da	кg/da	кg/da	% от N_0
N_0	661[d]	494[d]	519[d]	558	100	1223[d]	1061[d]	1068[d]	1117	100
N_{12}	777[c]	637[c]	679[c]	698	125,1	1329[d]	1294[c]	1340[b]	1321	118,3
N_{16}	851[b]	713[a]	774[a]	779	139,6	1417[c]	1342[c]	1373[b]	1377	123,3
N_{20}	918[a]	743[a]	804[a]	822	147,3	1559[a]	1491[a]	1425[a]	1492	133,6
N_{24}	849[b]	697[b]	764[b]	770	138,0	1341[d]	1267[c]	1358[b]	1322	118,4

**Evidence of differences at $P < 0.05$ if they do not have the same letters*

From the analysis of the degree of influence of the factors (Table 22) on the content and yield of dry matter in plants, it is established that these indicators are most strongly influenced by the harvesting stage (90.5%), followed by fertilization and the year, with 49.4% and 32.3%, respectively.

Table 22 Influence of the factors on the variation of dry mass yield

factors	SS	DF	MS	F	P	%
year	269138	2	134569	7,155	0,002880	32,3
phenostage	5397600	1	5397600	286,985	0,000000	90,5
fertilization	550621	4	137655	7,319	0,000308	49,4
year*stage	1769	2	884	0,047	0,954140	0,31
year*fertilization	15205	8	1901	0,101	0,998920	2
stage *fertilization	13576	4	3394	0,180	0,946737	2
year*stage *fertilization	13525	8	1691	0,090	0,999294	2

SS – the sum of squares, DF – degrees of freedom, MS – square of the mean value of the squares, F – the ratio between quantities, p – degree of evidence of the factor, % – degree of influence of the factor

It could be concluded on the basis of the analyses:

- ✓ The highest dry matter content in both harvest stages was reported in 2016 for the Musala variety and the milky ripe stage for the Atila variety. The differences in the dry matter values between 2014 and 2016 in the heading stage of the Atila variety are insignificant.
- ✓ The highest dry mass yields were obtained in 2016.
- ✓ The content and yields of dry matter in the milky ripe stage in both tested varieties exceed those in the heading stage.
- ✓ The lowest dry matter percentage and the lowest dry mass yields were obtained in the zero variants.
- ✓ Dry matter content and dry mass yield increase with increasing nitrogen fertilizer rates.
- ✓ The highest dry matter percentage and dry mass yields are obtained with the N20 fertilizer variant. There is no difference between the yields of the two tested varieties.
- ✓ The dry matter content in both tested varieties in the milky ripe stage is close to the optimal (30-35%) for obtaining better quality silage and more biogas.

5.4.2. Influence of nitrogen fertilization and harvesting stages on the morphological structure of dry plants

To determine the dry biomass and moisture in the whole plants and their organs after mowing and weighing in the field, they are dried.

The mass of a dry plant in the cultivars grown in the two stages of triticale harvest is reflected in Table 23.

In the first year of research and both stages of harvesting with heavier plants, the variety Atila stands out (from 3.46 g to 4.83 g; 5.44 to 7.38 g), and in the second and third, the variety Musala (from 3.34 g to 4.53 g; 5.32 g to 7.50 g; 4.21 g to 5.92 g; 7.31 g to 9.75 g), which also correlates with the magnitude of produces a dry mass.

Therefore, on average, for the three experimental years and in both harvesting stages, the plants of the Musala variety have a slightly larger mass than those of the Atila variety.

After heading, the mass of the plants continues to increase, and in the milky ripe stage, the amount increases significantly in terms of green and dry mass. In both varieties and harvesting stages, during the years of study and on average for the period, the mass of plants was the lowest in the non-fertilized variants.

Table 23 Dry mass of one plant (g)

Kg/da	Heading stage				Milky ripe stage			
	A plant weight, (g)				A plant weight, (g)			
	2014	2015	2016	Average	2014	2015	2016	Average
	Musala variety							
N_0	2,74	3,34	4,21	**3,43**	4,33	5,32	7,31	**5,65**
N_{12}	3,21	3,86	4,60	**3,89**	4,96	6,22	8,29	**6,49**
N_{16}	3,60	4,36	5,05	**4,34**	5,89	6,90	9,08	**7,29**
N_{20}	4,07	4,53	5,92	**4,84**	6,58	7,50	9,75	**7,94**
N_{24}	3.65	3,92	4,55	**4,04**	5,75	6,81	8,80	**7,12**
	Atila variety							
N_0	3,44	2,30	3,84	**3,19**	5,44	4,13	6,90	**5,49**
N_{12}	3,98	2,50	4,25	**3,58**	6,04	4,93	7,65	**6,21**
N_{16}	4,31	3,00	4,40	**3,90**	6,51	6,16	8,09	**6,92**
N_{20}	4,83	3,47	5,03	**4,44**	7,38	6,60	8,84	**7,61**
N_{24}	4,16	3,02	4,43	**3,87**	6,24	5,62	8,42	**6,76**

The data from the morphological analysis of the dry plants are reflected in tables 24 and 25.

In the heading stage of the Musala variety, the highest weight of the total plant weight is the stems, followed by the spikelets and leaves.

Therefore, the stems and spikelets have the largest share in forming the dry mass yield in this stage. The mass of stems varies from 1.77 g to 2.61 g; on spikelets, from 0.88 g to 1.35 g and on leaves from 0.78 g to 0.86 g.

Due to the slight differences in the values of the spikelets and leaves in the tested varieties, the indicators are arranged differently.

The slightly higher weight of leaves compared to spikelets in the Atila variety arranges the plant organs in contrast to the Musala variety in the following order: stem: leaves: spikelets. In the Atila variety, the stems and leaves have the largest share in the formation of the dry mass yield.

Masses of stems ranged from 1.62 g to 2.38, leaves from 0.80 g to 1.18 g, and spikelets from 0.77 g to 0.88 g).

Table 24 Morphological analysis of dry plants averaged over the growing period in the heading stage

kg/da	Weight per 1/plant (g)	Weight of the stem (g)	Spikelets weight/ a plant (g)	Leaf Weight/ a plant (g)
	Musala variety			
N_0	3,43	1,77	0,88	0,78
N_{12}	3,89	2,03	1,02	0,84
N_{16}	4,34	2,31	1,18	0,85
N_{20}	4,82	2,61	1,35	0,86
N_{24}	4,04	2,17	1,10	0,77
	Atila variety			
N_0	3,19	1,62	0,77	0,80
N_{12}	3,58	1,83	0,85	0,90
N_{16}	3,90	2,06	0,83	1,01
N_{20}	4,44	2,38	0,88	1,18
N_{24}	3,87	2,05	0,79	1,03

In the milky ripe stage, in both varieties tested, the value indicators are arranged in the following order: spikelets-stems-leaves, which is also confirmed by the research of Viorel et al. (2015). In this stage, the spikelets and stems have the largest share.

In the milky ripe stage, the plant mass of all tested parameters increases.

Also, in this stage, the spikelets and stalks of variety Musala (from 2.76 g to 4.15 g; 2.09 g to 2.54 g) have slightly higher values than variety Atila (from 2.59 g to 3.77 g; 2.00 g to 2.44 g).

The leaf weight values among the studied cultivars in the milky ripe stage were the lowest. The differences in their weights between the varieties are insignificant (in the Musala variety, they vary from 0.80 to 1.22 g, and in the Atila variety, from 0.90 g to 1.41 g).

In both varieties, regardless of the harvest stage, the values of these indicators are the lowest in unfertilized variants.

In the heading stage, the following values of the indicators were recorded for the Musala variety: weight of the stems - 1.77 g, the weight of the spikelets -0.88 g, and weight of the leaves -0.78 g. The same trend is observed in the variety Atila, in which the mass of the stems is 1.62 g, of the leaves - 0.80 g, and of the ears - 0.77.

Table 25 Morphological analysis of dry plants averaged over the growing period in the milky ripe stage

kg/da	Weight per 1/plant (g)	Weight of the stem (g)	Spikelets weight/ a plant (g)	Leaf Weight/ a plant (g)
	Musala variety			
N_0	**5,65**	2,76	2,09	0,80
N_{12}	**6,49**	3,21	2,35	0,93
N_{16}	**7,29**	3,71	2,46	1,12
N_{20}	**7,94**	4,06	2,66	1,22
N_{24}	**7,12**	3,63	2,38	1,11
	Atila variety			
N_0	**5,49**	2,59	2,00	0,90
N_{12}	**6,21**	3,01	2,08	1,12
N_{16}	**6,92**	3,40	2,26	1,26
N_{20}	**7,62**	3,77	2,44	1,41
N_{24}	**6,76**	3,34	2,16	1,26

Furthermore, in milky ripe, the lowest values of the indicators were registered for the non-fertilized variants. In the varieties Musala and Atila, the highest weight was recorded in the ears, 2.76 g; 2.59 g, followed by stalks, 2.09 g; 2.00 g, and the leaves, 0.80 g; 0.90g. Regarding the leaves, the differences between stages and varieties are insignificant.

In both varieties and stages, with an increase in fertilizer rates, the values of all indicators increase, while the highest one slightly decreases.

It can be seen from Figure 5 that, on average, for the period of the study, the stems, followed by the spikelets and leaves, have the highest share in the dry plants in the heading stage of the Musala variety.

In the variety Atila, due to the higher weight of the leaves, the percentage ratio of the organs follows the order: of stems, leaves, and spikelets.

The stems have the highest participation percentage in both varieties, and in the Musala variety (from 51.6% to 54.2%), it is higher than the Atila variety (from 50.8% to 53.6%).

In second place in terms of share in the Musala variety are the spikelets, which vary from 25.7% to 28.0%, and the leaves have the smallest share - from 22.7% to 17.84%.

In the Atila variety, after the stems in share, the leaves are ranked from 25.1% to 26.6%, and the ears are the lowest percentage in this variety.

In the Musala variety, the percentage participation of the stems and spikelets in the zero variants is the lowest (51.6%; 25.7%), and in the Atila variety in the stems and leaves (50.8%; 25.1%).

As the nitrogen fertilizer rate increases, the percentage participation of stems and spikelets in the Musala variety increases up to the N20 fertilizer rate.

Compared to the control, the proportion of stems increased by 2.6% and of spikelets by 2.3%, after which they slightly decreased.

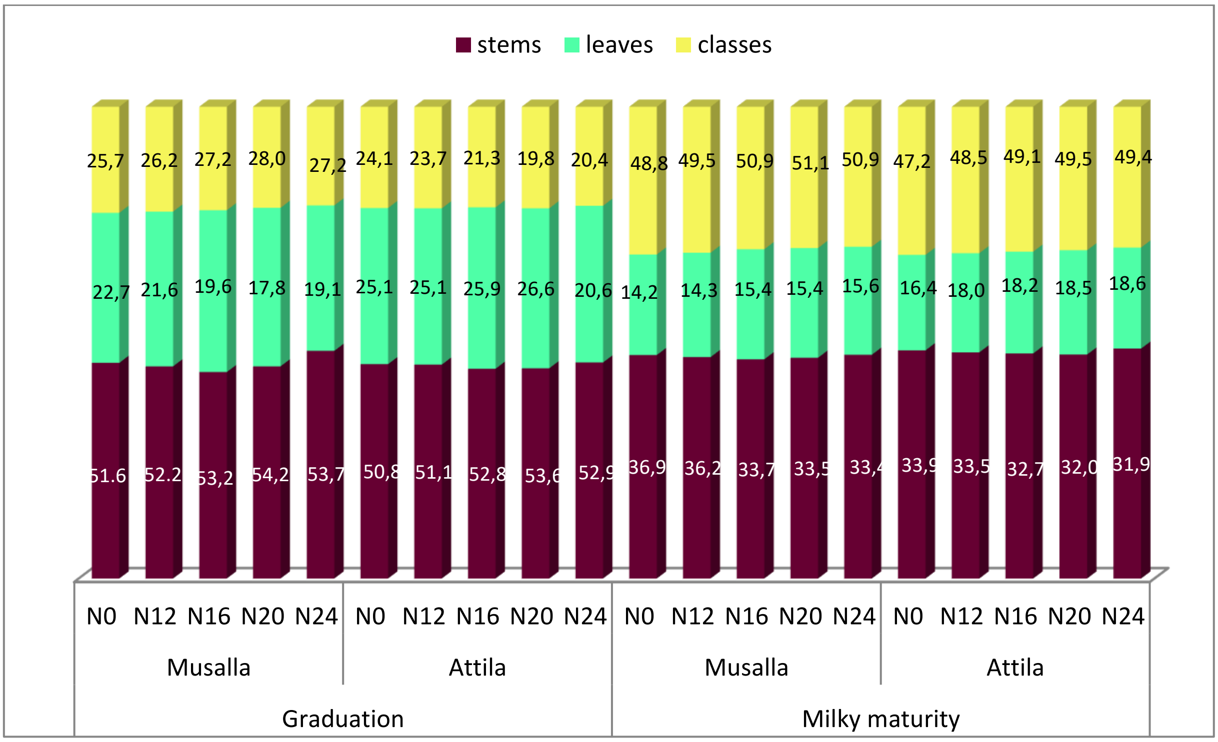

Figure 5 Percentage ratio of stems: leaves: spikelets in dry triticale plants in the stage of heading and milky ripe at different rates of nitrogen fertilization

While in the Atila variety, there is a tendency to increase the percentage participation to the variant fertilizer with N20 on the stems and leaves. Compared to the null variants, the proportion of stems increased by 2.8% and that of leaves by 1.5%. The percentage participation of the leaves in the Musala variety and the spikelets in the Atila variety decreased to the N20 fertilizer rate, from 22.7% to 17.8%; from 24.1 to 19.8%, after which they slightly increased. With an increase in the fertilizer rate, the leaf share in the Atila variety rises to the highest fertilizer rate.

In the milky ripe stage, in both cultivars and both harvest stages, the highest share falls on the ears that continue to grow, followed by the stems, and due to the almost final formation of the leaves, this share in the biomass decreases, which is also confirmed by the studies of Viorel et al., (2015).

In both varieties, the highest percentage of participation is in the grades, and in the Musala variety (from 48.8% to 51.1%), it is higher than that of the Atila variety (from 47.2% to 49.5%).

From the morphological structure of the plants made in this way, it is established that:

- ✓ The average dry weight of a plant has higher values in the milky ripe stage. Of the two tested varieties, an average higher dry mass per plant was recorded in the Musala variety, corresponding to the obtained dry mass yield.
- ✓ The highest share of the total mass of the plant in the heading stage of the Musala variety falls on the stems and spikelets, and in the Atila variety on the stems and leaves, while in the milky ripe stage in both varieties - on the spikelets and stems.
- ✓ Nitrogen fertilization has a positive effect on plant weight. The most substantial effect of it and the green mass was observed in the variants fertilized with N20 and the weakest in the non-fertilized variants.
- ✓ The percentage ratio of the organs of the dry plants in the heading stage follows the order: stems : spikelets: leaves in the Musala variety and stems: spikelets in the Atila variety, while in the milky ripe stage in both varieties, spikelets: stems: leaves.

5.4.3. Effect of nitrogen fertilization and harvesting stage s on green biomass silage yield

Cereals for silage, like maize, are easily ensiled without preservatives.
Optimum moisture for ensiling is easily reached by waiting for the appropriate stage of development. Silage is usually carried out in heading, milky to milky-waxy maturity, at 60-70% humidity. The silage is compacted most efficiently at this humidity, and significant surface losses and spoilage are avoided.

For the theoretical calculation of the amount of silage obtained from the green mass in our study, we used the data indicated in the publication of Krzystek et al. (2020).

The data in Tables 26 and 27 shows that silage yield follows the green mass yield because the percentage of total losses is the same (25%).

As a result, the highest yield of triticale silage for the Musala variety in both harvesting stages was obtained in 2016 (from 2805 kg/da to 3559 kg/da; from 2940 kg/da to 3848 kg/da), and for Atila variety in 2014 (from 2196 kg/da to 2936 kg/da; 2856 kg/da to 3524 kg/da).

The silage yield is the lowest in the non-fertilized variants. In the heading stage, they recorded average yields of 2135 kg/da for the Musala variety and 1929 kg/da for the Atila variety.
In both varieties (Musala and Atila) in the heading stage, the silage yield in the nitrogen fertilizer option 20 kg/da exceeds the control by 36%, respectively; 43.9%.

Table 26 Silage yield from the green mass of triticale in the heading stage and milky ripe in the Musala variety

N kg/da	Heading stage					Milky ripe stage				
	Years			Average	%	Years			Average	%
	2014	2015	2016			2014	2015	2016		
N_0	1532	2069	2805	2135	**100**	2625	2798	2940	2788	**100**
N_{12}	2004	2476	3069	2516	**117,8**	2682	3284	3455	3140	**112,6**
N_{16}	2043	2828	3396	2756	**129,0**	2870	3344	3591	3268	**117,2**
N_{20}	2295	2861	3559	2905	**136,0**	2913	3473	3848	3411	**122,3**
N_{24}	2010	2725	3242	2659	**124,5**	2867	3221	3380	3156	**113,1**

The silage yield was calculated based on the green mass yield at 25% losses (Krzystek L., 2020)

In the milky ripe stage, green mass and silage yield increase as the development stage s progresses.

Concerning the selected variants, the silage yields follow the same trend as in the heading stage but with higher values.

The average silage yields for the Musala variety varied from 2788 kg/da to 3411 kg/da and the Atila variety from 2594 kg/da to 3368 kg/da.
Moreover, in this stage, the yield is the lowest in the non-fertilized variants and the highest in the variant fertilized with nitrogen 20 kg/da.

Moreover, in this stage, the silage yield obtained in the variants fertilized with nitrogen 24 and 12 kg/da least exceeds the control. They exceed the control by 13.1%, 12.6% for the Musala variety, and 18.8%; 17.2% for the Atila variety. The silage yield values between the two variants are very close, with the difference between them being 16 kg/da for the Musala variety and 42 kg/da for the Atila variety.

Table 27 Silage yield from the green mass of triticale in the heading and milk stage maturity in the variety Atila

N kg/da	Heading stage					Milky ripe stage				
	Years			Average	%	Years			Average	%
	2014	2015	2016			2014	2015	2016		
N_0	2196	1859	1733	1929	**100**	2858	2526	2399	2594	**100**

N_{12}	2576	2389	2263	2409	**124,8**	3078	3053	2993	3041	**117,2**
N_{16}	2778	2666	2569	2671	**138,4**	3257	3120	3042	3140	**121,0**
N_{20}	2936	2757	2635	2776	**143,9**	3524	3429	3150	3368	**129,8**
N_{24}	2768	2655	2552	2658	**137,7**	3167	3074	3008	3083	**118,8**

The silage yield was calculated based on the green mass yield at 25% losses (Krzystek L., 2020)

5.4.4. Energy efficiency. Effect of nitrogen fertilization and harvesting stage s on biogas and methane production

The most important indicator when choosing energy crops is the net energy yield per hectare, which is mainly determined by the yield of biomass and the convertibility of biomass into biogas and methane. Biogas is a mixture of gases; on average, about 55% fall on flammable methane (CH4), the main component of biogas. For the production of biogas and methane, different types of biomass, green, dry, or silage plant raw materials can be used, therefore their values in various literature sources vary widely (Lehtomäki et al., 2006; Amon et al., 2007; Jenson et al., 2011; Griu, T. et al., 2012; Herrmann et al., 2011; Shilovoi., 2019).

The theoretical yield of biogas and methane was calculated based on the average yields of silage obtained from the green mass.

According to the publications of (Amon et al., 2006 and Braun., 2007), raw material yield is directly proportional to the amount of biogas and methane, which is confirmed by the data in our research.

Depending on the chosen options, in the Musala variety's heading stage, the amount of biogas varies from 4057 Nm3 ha-1 to 5622 Nm3 ha-1. In contrast, in the milky ripe stage, the biogas yields increase proportionally to the silage yields. Higher biogas values were recorded in this stage, from 5855 Nm3 ha-1 to 7283 Nm3 ha-1 (tables 28 and 29).

The lowest biogas yields are obtained from the unfertilized variants - 4057 Nm3 ha-1; 5855 Nm3 ha-1.

With an increase in the nitrogen fertilizer rate, biogas yields increase. In both harvesting stages, the highest yield is obtained with the nitrogen fertilizer variant 20 kg/da, 5622 Nm3 ha-1; 7283 Nm3 ha-1 obtained from 29.5 t/ha; 34.11 t/ha silage. In the variant with nitrogen fertilizer 20 kg/da, the highest additional biogas yield is obtained compared to the control (1565 Nm3 ha-1 in the heading stage and 1428 Nm3 ha-1 in the milky ripe stage).

With the fertilizer option with the highest nitrogen fertilizer rate, N24 (5052 Nm3 ha-1; 6628 Nm3 ha-1), the biogas yield is lower than the previous ones, N20 and N16 but higher than the fertilizer option with N12 (4780 Nm3 ha-1; 6594 Nm3 ha-1).

The main component of biogas is flammable methane (CH4). According to Gerlach et al. (2013), its content in cereals is 53-56%, on average about 55%.

Table 28 Biogas and methane production from silage at different levels of fertilization and harvest stages in the Musala variety

N kg/da	Retraction stage s					
	Graduation			Milky maturity		
	Average yield Silage t/ha	Biogas (Nm^3 ha^{-1})	Methane (Nm^3 ha^{-1})	Average yield Silage t/ha	Biogas (Nm^3 ha^{-1})	Methane (Nm^3 ha^{-1})
N_0	21,35	4057	2231	27,88	5855	3220
N_{12}	25,16	4780	2629	31,40	6594	3226
N_{16}	27,56	5236	2880	32,68	6863	3775
N_{20}	29,05	5622	3092	34,11	7283	4006

N_{24}	26,59	5052	2779	31,56	6628	3645

The biogas and methane yields were calculated based on the average silage yields from green mass at 190 m3 /t-1 and 210 m3 /t-1 in the heading and milky ripe stage, with a methane content in biogas of 55% (Weiland., 2010).

In the Musala variety, the methane yield varies from 2231 Nm3 ha-1 to 3092 Nm3 ha-1, in the heading stage and from 3220 Nm3 ha-1; 4006 Nm3 ha-1 in the stage of milky ripe, which is close to the yields obtained by Amona et al. (2007) for cereal crops harvested at milky ripe (3200-4500 m3 N/ha).

The amount of biogas and methane yields for the Atila variety follows the same trend as for the Musala variety but with lower values.

Moreover, with this variety, the lowest biogas yields are obtained from the non-fertilized variants, 3665 Nm3 ha-1; 5447 Nm3 ha-1.

Depending on the selected options in the heading stage of the Atila variety, the amount of biogas varies from 3665 Nm3 ha-1 to 5272 Nm3 ha-1. In contrast, in the milky ripe stage, the yields increase proportionally to the silage yields and reach 5447 Nm3 ha-1 - 7073 Nm3 ha -1.

Furthermore, the lowest biogas yields are obtained from the non-fertilized variants with the Atila variety, 3665 Nm3 ha-1; 5447 Nm3 ha-1.

Of the fertilized variants with the lowest biogas yields are the variants with N12, 4577 Nm3 ha-1; 6386 Nm3 ha-1.

Fertilization with 20 kg/da nitrogen had the most substantial effect on the amount of biogas in both harvesting stages, where the yield exceeded the control by 1609 Nm3 ha-1; 1626 Nm3 ha-1.

The methane yields of the Atila variety follow the same trends as those of the Musala variety. Moreover, for this indicator, the values of the Musala variety are higher than those of the Atila variety. In the heading stage, methane yields ranged from 2016 Nm3 ha-1 to 2901 Nm3 ha-1, and in milky ripe, from 2996 Nm3 ha-1 to 3890 Nm3 ha-1.

Table 29 Production of biogas and methane from silage obtained at different levels of fertilization and harvest stages in the Atila variety

N kg/da	Retraction stage s					
	Graduation			Milky maturity		
	Average yield Silage t/ha	Biogas (Nm^3 ha^{-1})	Methane (Nm^3 ha^{-1})	Average yield Silage t/ha	Biogas (Nm^3 ha^{-1})	Methane (Nm^3 ha^{-1})
N_0	19,29	3665	2016	25,94	5447	2996
N_{12}	24,09	4577	2517	30,41	6386	3512
N_{16}	26,71	5075	2701	31,40	6594	3627
N_{20}	27,76	5274	2901	33,68	7073	3890
N_{24}	26,58	5050	2778	30,83	6474	3561

The biogas and methane yields were calculated based on the average silage yields from green mass at 190 m3 /t-1 and 210 m3 /t-1 in the heading and milky ripe stage, with a methane content in biogas of 55% (Weiland., 2010).

In the theoretical calculation of the yields of silage, biogas, and methane, it is established that:

- ✓ Higher silage, biogas, and methane yields are obtained in the milky ripe stage. Fertilization with 20 kg/da of nitrogen substantially affects the amount of biogas and methane, in which yields reach the highest values for the Musala variety - 7283 Nm3 ha-1; 4006 Nm3 ha-1.

7. CONCLUSIONS

Based on the experimental work performed and the results obtained, the following conclusions could be drawn:

1. Varietal differences in the phenological development of the tested varieties of triticale, Musala, and Atila, under the conditions of the Plovdiv region, grown for green mass, were found.
2. It was found that higher mean values of leaf area and intensity of transpiration in both harvest stages were recorded in cultivar Atila. The net photosynthesis rate and plastid pigments' content were higher in the Musala variety.
3. As the vegetation progresses, the values of the leaf surface, the rate of net photosynthesis, and the content of plastid pigments in the leaves decrease, and the intensity of transpiration increases. Nitrogen fertilization has a positive effect on physiological parameters.
4. Higher values of the leaf surface in both tested varieties and harvest stages were recorded in the N20 fertilization option. In contrast, in the intensity of photosynthesis and transpiration indicators, in the heading stage of both varieties, the highest values were recorded when fertilized with N20 and in the milky ripe stage when fertilized with N16.
5. In terms of yield, there is a solid varietal differentiation. In the wetter 2014, higher yields were obtained with the Atila variety, while in the drier 2015. and 2016 for the Musala variety. Higher yields of green mass are obtained in the milky ripe stage. Fertilized variants are superior in yield to unfertilized variants. The variants with N20 nitrogen stand out with the highest yields - 4548 kg/da for the Musala variety and 4490 kg/da for the Atila variety. Under all fertilization options, average yields exceeded the control in both cultivars, indicating that triticale has excellent potential for inclusion in sustainable energy crop rotations for biomass production under less favorable growing conditions.
6. The highest effect of fertilization (expressed by the green mass obtained from 1 kg of applied nitrogen) in the leveling stage is obtained with the variant fertilized with 16 kg/da of nitrogen (51.0 kg/da; 61.8 kg/da) and in the stage of milky ripe in the fertilizer variant with 20 kg/da of nitrogen (41.6 kg/da; 52.1 kg/da). The highest additional yield was reported for the N20 nitrogen fertilizer variant in the heading stage - 1014 kg/da and 1129 kg/da, respectively.
7. The Musala variety has a denser crop and higher productive tillers in both harvest stages. Fertilization with nitrogen fertilizers increased the number of spikelets-bearing stems formed per unit area in both stages: from 12.7% and 13.4% at the lowest level of fertilization (N12) to 46.8% and 43.1% at the highest - the high level (N24) of fertilization, as well as the percentage of productive tillers.
8. The values of structural elements in the milky ripe stage have higher values, except for the number of leaves. As a result, on average, over the three years of the study, the Musala variety formed 8.9% and the Atila variety 11.6% higher yields during the milky ripe stage. The values of all the tested indicators in the variants fertilized with nitrogen fertilizer exceeded the control on average in the heading stage by 21.3% in the Musala variety and 29.0% in the Atila variety, and the milky ripe stage - 13.1% and 17.4%.
9. The morphological analysis of the plants establishes that the percentage ratio of the organs of the fresh plants in the heading stage at different nitrogen fertilization rates follows the order - stems: leaves: spikelets, and in the milky ripe stage - stems: spikelets: leaves. The percentage ratio of the organs of the dry plants in the heading stage follows the order - stems: spikelets: leaves in the Musala variety and stems: spikelets in the Atila variety, while in the milky ripe stage in both varieties, spikelets: stems: leaves.
10. In the two stages of harvesting, both in the fresh and in the dry mass, the highest share is occupied by the stems.
11. The dry matter content in both tested varieties in the milky ripe stage has values close to the optimal (30-35%) for obtaining better quality silage and a more significant amount of biogas. The content and yields of dry matter are higher in the milky ripe stage. The highest dry matter content and the highest dry mass yields were obtained with the N20 fertilizer option. The yields obtained in this stage and this variant for the two tested varieties are almost the same - 1484 kg/da for the Musala variety and 1492 kg/da for the Atila variety.
12. Silage yield is calculated at 25% losses. Higher silage yields were reported in the milky ripe stage, from 2788 kg/da to 3411 kg/da in the Musala variety and from 2594 kg/da to 3368 kg/da in the Atila variety. Fertilization with 20 kg/da nitrogen substantially affects the amount of silage, where yields reach the highest values.

13. Higher yields of biogas (from 5855 Nm3 ha-1 to 7283 Nm3 ha-1) and methane (from 3220 Nm3 ha-1 to 4006 Nm3 ha-1) can be obtained at the milky ripe stage in the Musala variety. The strong effect on the amount of biogas and methane is exerted by fertilization with 20 kg/da of nitrogen, in which yields reach the highest values for the Musala variety - 7283 Nm3 ha-1; 4006 Nm3 ha-1.
14. The Atila variety has a higher crude protein content on average for the period, and the Musala variety has a higher crude fiber and fat content variety. The BEV values are almost identical, with a slight preponderance of the Atila variety. As the development stages progress, the crude protein content decreases at the expense of the other components, and with an increase in nitrogen fertilizer rates, the opposite trend is observed. According to literature sources, crude protein, fiber, fat, and BEV indicators are within the optimal range for obtaining quality silage and a high amount of biogas in both varieties and harvesting stages.
15. The biogas and methane yields in triticale, apart from biomass yield, depend significantly on its chemical composition. Crude protein and crude fiber contribute the most to biogas and methane production from triticale. All nitrogen-fertilized variants exceeded the control ones in terms of yield. The highest yield of crude protein (187.3 kg/da CU), crude fiber (408.4 kg/da), crude fat (19.06 kg/da), and BEV (901.9 kg/da) obtained in the milky ripe stage of the Atila variety, in the variant fertilized with 20 kg/da of nitrogen. However, the differences are significant only in the production of raw protein, and the values with the other chemical parameters are almost the same as those of the Musala variety.

8. CONTRIBUTIONS

8.1. Scientific and theoretical contributions

1. Varietal differences in the phenological development of the tested triticale, Musala and Atila, grown for green mass, were found. The duration and the necessary temperature sum for the interstage 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 Atila and Musala varieties for green mass in the Plovdiv region and their change under the influence of nitrogen fertilization and harvesting stages were established.
3. The Atila variety was superior to the Musala variety in nitrogen assimilation efficiency, being more energy efficient and 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 established.
5. Silage, biogas, and methane yields were theoretically calculated for the triticale varieties, Musala and Atila.

8.2. Scientific and applied contributions

1. The genotypic specificity of two modern Bulgarian triticale varieties has been established for the nitrogen fertilization level and the harvesting stages for green mass under Central South Bulgaria.
2. It was established that in heavy rainfall during the heading 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 for yields of green mass in drier years for Central South Bulgaria.
4. A stable tendency to increase green and dry mass yields 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/day. Using a high nitrogen rate of 24 kg/day 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.

Publications of Hristina Atanasova Nedeva in connection with the dissertation

1. Nedeva, H., Ivanova, R., & Yancheva, H. (2016). Plastid pigments quantity and some physiological parameters related to photosynthetic processes in triticale grown for green biomass. Agricultural Science and Technology, 8(2), 117-120

2. Nedeva, H., Yancheva, H., & Ivanova, R. (2016). Growing triticale biomass at various nitrogen fertilization rates and harvesting stages. In VII International Scientific Agriculture Symposium," Agrosym 2016", 6-9 October 2016, Jahorina, Bosnia and Herzegovina. Proceedings (pp. 232-237). The University of East Sarajevo, Faculty of Agriculture.

3. Nedeva, H. (2016). Influence of triticale structural elements on the green mass yield in dependency on the nitrogen rate and the harvesting stage. Scientific Papers. Series A. Agronomy, Vol. LXIII, No. 2, p. 165-171. 2020 ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

Effect of nitrogen fertilization and harvest time on productivity and quality of triticale green biomass used for energy purposes

The great interest in many crops as sources for obtaining biofuels is the continuous reduction of fossil raw materials, the maximum of which will occur by 2030. That is why the production of biofuels as an alternative energy source is particularly relevant at present. In connection with world trends, the new development strategy of the European Union, part of which is the "Green Deal," and in connection with the use of alternative methods for the production of green energy, crops such as triticale are finding more and more space.

The great potential of the culture in this direction is due to the unique combination of the good qualities of the species from which it originates, namely - rye and wheat. The culture fits very well in the circular bioeconomy, which is increasingly being imposed recently, where the main share of crop production is the production of raw materials. Our country's average yields of green mass, depending on the variety and climatic factors of the respective region, ranging from 3 to 7 t/day, is equivalent to 1000-1200 kg/day of dry matter.

Based on the derived experimental work and the obtained results, varietal differences in the phenological development of the tested triticale, Musala, and Atila, grown for green mass, were established. The duration and the necessary temperature sum for the interstage periods and their vegetation period under different weather conditions during the years of research for the conditions of the Plovdiv region were determined. The physiological characteristics of triticale in Atila and Musala varieties for green mass in the Plovdiv region and their change under the influence of nitrogen fertilization and harvesting stages were established.

The Atila variety is superior to the Musala variety in terms of nitrogen uptake efficiency, being more energy efficient, and obtaining higher yields with an input nitrogen consumption of 1 kg. In terms of yield, there is a solid varietal differentiation. In the wetter 2014, higher yields were obtained with the Atila variety, while in the drier 2015. and 2016 for the Musala variety. Higher yields of green mass are obtained in the milky ripe stage. Fertilized variants are superior in yield to unfertilized variants. The variants with nitrogen N20 stand out with the highest yields -4548 kg/da for the Musala variety and 4490 kg/da for the Atila variety. Under all fertilization options, average yields exceeded the control in both cultivars, indicating that triticale has excellent potential for inclusion in sustainable energy crop rotations for biomass production under less favorable growing conditions. In the theoretical calculation of silage, biogas, and methane yields, it is established that higher silage, biogas, and methane yields are obtained in the milky ripe stage. Fertilization with 20 kg/da of nitrogen substantially affects the amount of biogas and methane, in which yields reach the highest values for the Musala variety - 7283 Nm3 ha-1; 4006 Nm3 ha-1.