

Impact of agro-ecological conditions and fertilization on yield and quality of triticale on pseudogley soil

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Abstract

The results of the study of the influence of fertilization and calcification on the yield and yield components of winter triticale grown on low pH soil are presented in this paper. Five variants of fertilization were tested during three growing seasons. Trial treatments included different fertilization variants: V1-control, V2-N120, V3-N120P80K60, V4-N120P80K60 + 5 t ha⁻¹ of lime and V5-N120P80K60 + 5 t ha⁻¹ of lime + 30 t ha⁻¹ of manure. The results of the research showed that all yield components responded positively to the application of mineral nutrition by changing the production characteristics and grain quality. The variant with the combined application of NPK, lime and manure had the greatest positive effect on all tested parameters affecting triticale productivity. During the study, the highest yield of triticale 5.826 t ha⁻¹ was obtained on the fertilization variant with the combined application of lime, manure and NPK fertilizers. The achieved increase in the grain yield of triticale was significant compared to the control and the NPK variant of fertilization. Highly significant positive dependencies were found between the grain yield with 1000-grain weight, and test weight and significant positive dependencies were found between 1000-grain weight and test weight. The research highlighted the impact of different fertilizer treatments on the yield and grain quality traits of winter triticale.

Keywords: fertilizer; grain quality; protein content; triticale; yield

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Introduction

Triticale is a new type of small grain, which was created by human breeding and selection. By crossing several types of wheat and rye, several types of triticale were obtained (Milovanovic *et al.*, 2014; Camerlengo and Kiszonas, 2023). Great genetic variability is important for the breeding process itself, as well as the way of introducing desirable genes, which, with the selection of genotypes with good performance, results in the creation of varieties of good agronomic and technological quality (Đurić *et al.*, 2015; Kirchev and Georgieva, 2017; Losert *et al.*, 2017). In many countries, including ours (The Republic of Serbia), the areas under triticale, as well as the total production, are constantly increasing from year to year. The production of triticale in the Republic of Serbia varies depending on the price and demand on the market, as well as on the agroecological conditions at the time of establishment of the crop (Biberdžić *et al.*, 2017). Year after year, production of this type of triticale takes place in very unfavourable conditions, especially for primary producers. Wide variations in the prices of inputs and finished products (mercantile grain), uncertain market placement and the possibility of storage represent production risk factors (Babić *et al.*, 2023). To date, a large number of high-yielding and high-quality triticale varieties have been created in the Republic of Serbia (Djekic *et al.*, 2011) influencing an increase in yield per hectare. The massive introduction of low-stemmed varieties into production and the improvement of technological production, i.e. increasing the amount of mineral fertilizers have contributed to the increase in the yield of triticale (Madić *et al.*, 2018).

The yield and quality of small grains depend mostly on balanced mineral nutrition. The mineral nutrition of small grains depends on the type of soil, climatic factors of the region, and other agroecological factors (Jelić *et al.*, 2013; Rajičić *et al.*, 2020; Ugrenović *et al.*, 2021; Laidig *et al.*, 2022). On soils with an acid reaction, the mineral nutrition of triticale shows certain specificities. New triticale varieties have significantly higher fertility potential, however, their requirements in terms of mineral nutrition are significantly higher (Dogan *et al.*, 2011; Ivanova and Tsenov, 2014; Kucukozdemir *et al.*, 2019; Derejko *et al.*, 2020).

In winter, triticale uses large amounts of mineral elements during the growing season and has high requirements in regard to the soil fertility (Kendal *et al.*, 2016; Flajšman *et al.*, 2020). Triticale absorbs the most nitrogen, potassium, phosphorus, sulfur, magnesium, and calcium from the soil (Bielski and Falkowski, 2017). To achieve a high yield of triticale grains, depending on the type of soil, in the Republic of Serbia, nitrogen is mostly used in the amount of 80 to 120 kg ha⁻¹ (Terzic *et al.*, 2018; Lalević *et al.*, 2019; Rajičić *et al.*, 2020). Nitrogen, of all elements of mineral nutrition, plays the greatest role in increasing the yield (Janušauskaite, 2014; Gerdzhikova *et al.*, 2017; Bielski *et al.*, 2020). Soil acidity is the most common limiting factor in the production of small grains in the Republic of Serbia. In Serbia, primarily in its central part, slightly acidic, acidic and extremely acidic soils are significantly represented, with over 60% of the total arable land (Jelić *et al.*, 2015; Gudžic *et al.*, 2019). Of all applied agrotechnical measures, the application of mineral fertilizers, primarily nitrogen, has enabled an increase in grain yield and total amounts of protein per unit area in new varieties (Đekić *et al.*, 2014; Biberdžić *et al.*, 2017; Terzic *et al.*, 2018). Before the energy crisis, the price of mineral fertilizers was economically acceptable, so there was a tendency to apply increased quantities to the level where varieties were able to react by increasing yields. Recently, there is a need to formulate a fertilization system that will be adapted to climatic and soil conditions, meaning that there is no single formula applicable in every cultivation area (Biberdžić *et al.*, 2012). However, there are universal, basic principles of fertilization and mineral element requirements of triticale plants that must be respected if full exploitation of the genetic potential of cultivars and cultivation sites is desired (Bielski, 2015; Kucukozdemir *et al.*, 2018; Lalević *et al.*, 2022). Inadequate application of fertilizers, regardless of the requirements of plants and soil fertility, leads to the accumulation of unused nutrients in the soil, which poses a threat to the overall ecosystem and threatens the production of healthy and safe food (Biberdžić *et al.*, 2017; Terzic *et al.*, 2018; Rajičić *et al.*, 2020; Babić *et al.*, 2023).

The goal of the study was to examine the impact of different combinations of mineral and organic fertilizers on grain yield and quality, as well as to determine their adequate application in order to achieve the most profitable and economically justified production of triticale on soils with a low pH value.

Materials and Methods

Experimental design

The experiment was set up in the Secondary Agricultural Chemical School "Dr. Djordje Radic" in Kraljevo (Republic of Serbia) at 43°43'00''N and 20°40'60''E, at an altitude of 198 m, during three production years, with the aim of analysing the yield and quality of triticale grains.

The experiment was set up according to the random block system in five repetitions, with the size of the elementary plot 500 m² (25 × 20 m). The experiment was set up on the pseudogley type soil. The preceding crop in both years was corn. Sowing was done with a small mechanical seeder in both years in the middle of the optimum agrotechnical period (12 October, 2015; 26 October, 2016, and 17 October, 2017), at an inter-row distance of 12.5 cm and 3 cm in a row. The winter triticale cultivar used in the experiment was 'Favorit', which was created at the Center for Small Grains in Kragujevac (The Republic of Serbia). The amount of seeds per square meter was 600 germinated seeds per m², depending on the characteristics of the variety.

The research included the control (V1) and four treatments of fertilizers: NPK (V2); NPK and 5 t ha⁻¹ of lime (V3), NPK and manure 20 t ha⁻¹ (V4) and NPK and lime 5 t ha⁻¹ and manure 20 t ha⁻¹ (V5). In all fertilizer variants, in addition to nitrogen in the form of KAN (calcium-ammonium nitrate, 27% N) added in the amount of 120 kg ha⁻¹, also phosphorus in the form of super-phosphate was added in the amount of 80 kg ha⁻¹, and potassium in the form of 60% potassium salt in the amount of 60 kg ha⁻¹. NPK fertilizer formulation was used in the experiment in pre-sowing soil preparation. The total amounts of phosphorus, potassium, and manure together with one-third of nitrogen were spread by hand on the ploughing surface before the pre-sowing preparation of the soil. In early October 2015, non-hydrated CaO lime, 99% pure, in the amount of 5 t ha⁻¹ was applied for calcification of the soil and incorporated at a depth of 25 cm. During the growing season of triticale, standard care measures were applied in the trial. At the beginning of the intensive growth of triticale plants, in the 2-3 leaf phase (phase of full rooting), at the beginning of March, AN (ammonium nitrate, 33% N) was applied as a supplement.

The harvest was carried out manually in the phase of full maturity in the middle of June (dates of harvesting 10 June 2016, 6 June 2017 and 28 June 2018), and the measured grain yield was corrected to 14% moisture. The following properties were analyzed: grain yield (GY), 1000-grain weight (TGW), test weight (TW) and protein content (PC) were quantified. The nitrogen content in the grain of the tested triticale cultivars was determined by the Kjeldahl method, whereby the obtained values were multiplied by a coefficient of 6.25.

Soil analysis

The soil on which the survey was carried out was the pseudogley type with a low pH value (pH (KCl) = 4.46) and unfavourable physical, chemical and microbiological properties. The soil is well supplied with humus (2.18%) with a low content of available phosphorus (< 10 mg 100 g⁻¹ soil) and a medium content of readily available potassium (13 - 18 mg 100 g⁻¹ soil).

Statistical analysis

Experimental data were analysed by descriptive and analytical statistics using the statistics module Analyst Program GenStat (2013) for PC/Windows 7. All evaluations of significance were made on the basis of the ANOVA test at 5% and 1% significance levels. Relative dependence was defined through correlation

analysis (Pearson's correlation coefficient) and the coefficients obtained were tested at the 5% and 1% levels of significance.

Meteorological conditions

The experiment was set up in the vicinity of Kraljevo (Republic of Serbia) at 43°43'00'' N and 20°40'60'' E, at an altitude of 198 m. The experiment was set up according to a random block system in the period from 2015-2018. The weather conditions are shown in Figures 1-3.

The weather conditions in the examined area during the three growing seasons had certain deviations in relation to the multi-year average (1981-2010) characteristic of the area of Kraljevo (Figure 1).

The production year 2015/16 showed very favorable agrometeorological conditions. Thanks to large amounts of precipitation at the beginning and end of September, the humidity of the surface and deeper layers of arable agricultural soil was very favourable for the preparation and sowing of triticale. The period from October 2015 to the end of June 2016 was warmer compared to the multi-year average (Figure 1).

The deviation of the mean annual temperature was 1.24 °C, with a slightly higher rainfall (651 mm) compared to the multi-year average (539.4 mm). During the year, there were no significant extreme weather deviations from the average characteristics for the examined area that would pose risk for the production of triticale.

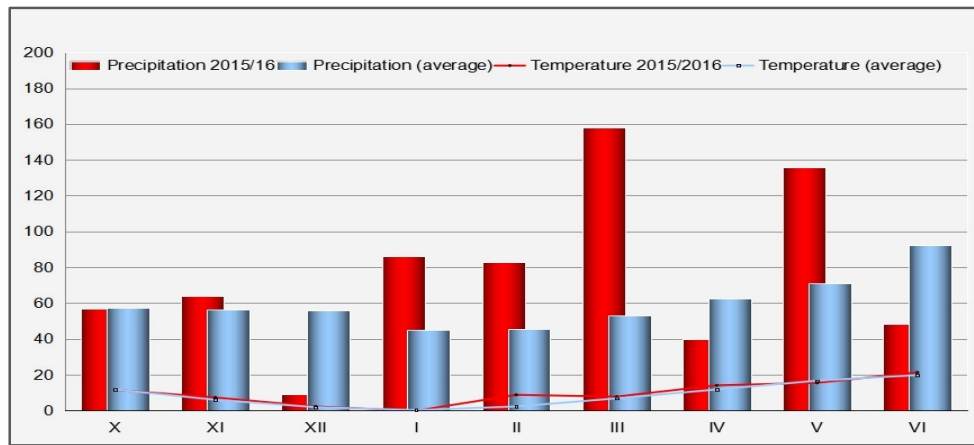


Figure 1. Temperatures (°C) and precipitation (mm) in Kraljevo, Republic of Serbia (2015-2016) and long-term average (1981-2010)

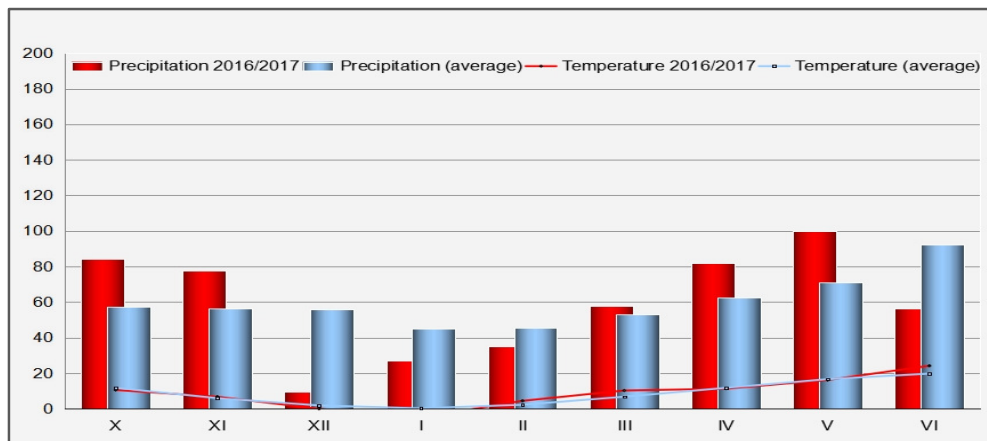


Figure 2. Temperatures (°C) and precipitation (mm) in Kraljevo, Republic of Serbia (2016-2017) and long-term average (1981-2010)

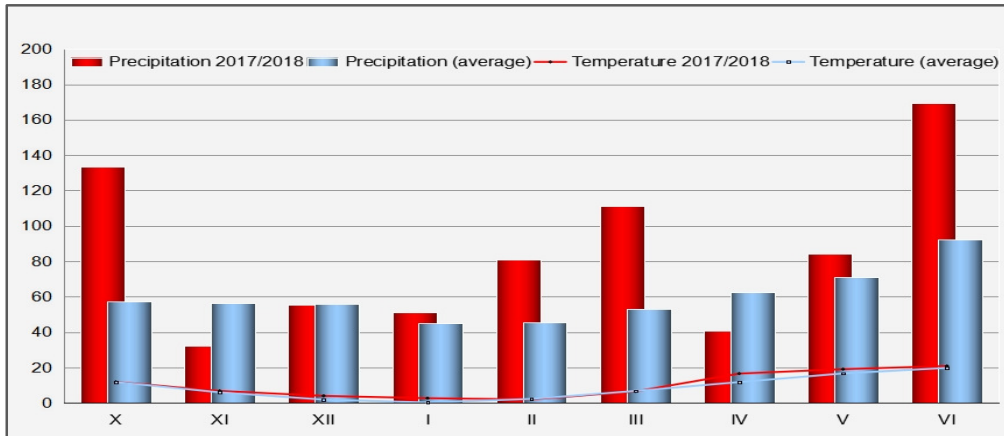


Figure 3. Temperatures (°C) and precipitation (mm) in Kraljevo, Republic of Serbia (2017-2018) and long-term average (1981-2010)

The production year 2016/17 was warmer by 0.17 °C, with a lower rainfall (529.4 mm) compared to the multi-year average (Figure 2). The growing season of 2016/17 was unfavourable for many agricultural crops from the point of view of agrometeorological conditions. Certain unfavourable impacts on the production of triticale were: late spring frosts, snow at the end of April, and occurrences of drought and heat waves in the summer, which affected the reduction of yield and lower quality of triticale grains.

In the production year 2017/18, in the period from 1 October, 2017 to 30 June, 2018, an average of 758.7 mm was recorded in the area of Kraljevo, which is by 40% more than the multi-year average (Figure 3).

Unfavourable weather conditions in the month of June 2018 due to heavy rainfall caused triticale crops to lie down, which led to losses in grain quality and overall triticale grain yield. The yields of the winter triticale crop in this production year were slightly higher than in the 2016/17 production year and slightly lower compared to the 2015/16 production year, with poorer quality of the grain.

Results

Grain yield

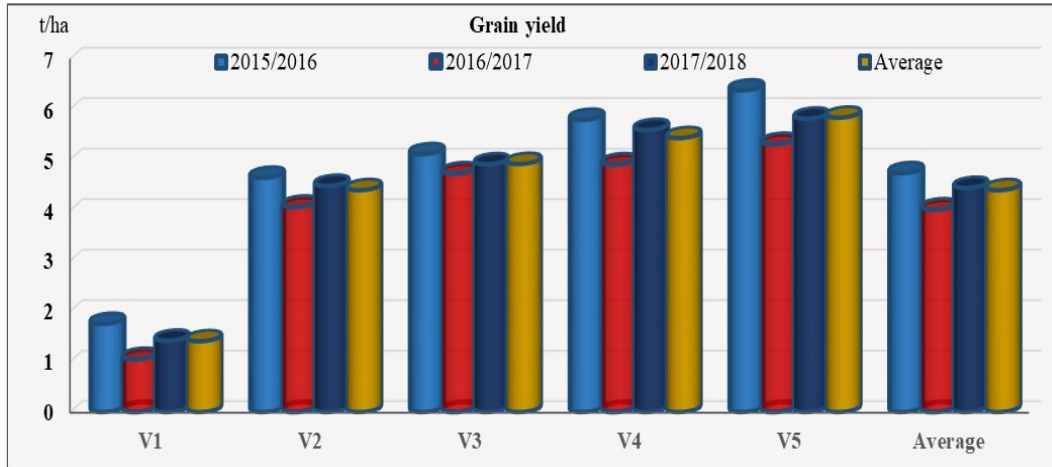
The average values of grain yield with different variants of fertilization of winter triticale crops, during the three production years, are shown in Table 1.

In terms of yield, differences were found between the examined growing seasons and fertilization variants. The average yield of triticale for all investigated growing seasons and fertilization variants during the three-year trial was 4.390 t ha⁻¹. During the investigated period, the highest average yield of triticale grains of 5.826 t ha⁻¹ was achieved in variant V5, fertilized with mineral NPK fertilizer in combination with lime and manure.

The highest average grain yield of 4.727 t ha⁻¹ for triticale was achieved in the first growing season of 2015/16 and was higher by 721 kg ha⁻¹ compared to the yield in the production year 2016/17 and by 291 kg ha⁻¹ higher compared to the yield in the production year 2017/18, which is the result of the proper distribution of precipitation during the first vegetation period. The highest grain yield in the production year 2015/16 was recorded in variant V5 (6.366 t ha⁻¹), fertilized with NPK, lime and manure, and the lowest in variant V1 (1.743 t ha⁻¹), which was not fertilized, Figure 4.

Table 1. Grain yield of winter triticale in Kraljevo, Serbia

Fertilization	Years						Average	
	2015/16		2016/17		2017/18			
	\bar{x}	S	\bar{x}	S	\bar{x}	S	\bar{x}	S
V1	1.743	0.349	1.046	0.212	1.410	0.476	1.399	0.446
V2	4.636	0.504	4.060	0.414	4.476	0.412	4.391	0.483
V3	5.104	0.321	4.721	0.359	4.904	0.245	4.910	0.331
V4	5.784	0.395	4.899	0.274	5.584	0.256	5.422	0.488
V5	6.366	0.504	5.304	0.206	5.808	0.356	5.826	0.568
Average	4.727	1.681	4.006	1.590	4.436	1.652	4.390	1.646

**Figure 4.** Effect of fertilization on grain yield of winter triticale in Kraljevo, Republic of Serbia

The average yield of triticale during the second growing season was 4.006 t ha⁻¹. The highest grain yield in the production year 2016/17 was recorded in the variant V5 (5.304 t ha⁻¹) fertilized with NPK, lime and manure, but a good yield was also achieved in variant V4 which was fertilized with NPK and manure (4.899 t ha⁻¹).

During the production year 2017/18, the average yield for all tested fertilization variants was 4.436 t ha⁻¹. During the study period, the highest average grain yield was obtained in the V5 variant, where a combination of NPK with lime and organic fertilizer was applied (5.808 t ha⁻¹), and the lowest in the control variant (1.410 t ha⁻¹).

The analysis of yield variance of winter triticale of the tested fertilization variants during three growing seasons is shown in Table 2. Based on the variance analysis data, it can be concluded that no significant influence of year on grain yield was established between the studied growing seasons. The influence of fertilization on the yield of triticale in the examined different varieties of fertilization was highly significant ($F_{exp} = 209.670^{**}$). Also, the interaction between the examined vegetation seasons and the fertilization variants during the research had a highly significant effect on the yield of triticale ($F_{exp} = 103.114^{**}$).

Table 2. The analysis of variance for grain yield in Kraljevo, Republic of Serbia

Effect	Df	Mean sqr Effect	Mean sqr Error	F	p-level
Year, (Y)	2. 72	3.28895	2.694038	1.22082	0.301 ^{ns}
Fertilization, (F)	4. 70	46.27484	0.220703	209.6699	0.000 ^{**}
Year x Fertilization, (YxF)	14. 60	13.75328	0.133379	103.1144	0.000 ^{**}

^{ns}non significant; ^{*}significant at 0.05; ^{**}significant at 0.01;

Thousand grain weight

The average values of the 1000-grain weight of the tested fertilization variants in winter triticale, during the three production years, are shown in Table 3.

Table 3. Thousand grain weight of winter triticale in Kraljevo, Republic of Serbia

Fertilization	Years						Average	
	2015/16		2016/17		2017/18			
	\bar{x}	S	\bar{x}	S	\bar{x}	S	\bar{x}	S
V1	41.80	1.966	38.00	2.367	41.00	1.875	40.27	2.564
V2	43.12	1.383	40.32	2.020	42.12	1.735	41.85	2.003
V3	43.42	1.055	40.22	0.377	41.82	1.069	41.82	1.585
V4	43.78	1.281	40.34	0.297	42.58	1.408	42.23	1.799
V5	45.36	2.023	40.34	0.910	43.56	1.940	43.09	2.665
Average	43.50	1.864	39.84	1.637	42.22	1.727	41.85	2.298

The grain of triticale in the tested fertilization variants had a good 1000-grain weight, especially in the first and third production years. The average 1000-grain weight of triticale for all investigated growing seasons and fertilization variants during the research period trial was 41.85 g. During the three years, the highest average 1000-grain weight of triticale of 43.09 g was achieved in variant V5, where a combination of NPK with lime and organic fertilizer was applied and the lowest in the control variant (40.27 g).

The 1000-grain weight of triticale in 2015/16 was significantly higher than in 2016/17 year (Table 3). The highest average 1000-grain weight of triticale of 43.50 g was achieved in the production year 2015/16. The highest 1000-grain weight in the first growing season was recorded in variant V5 (45.36 g), fertilized with NPK, lime and manure, and the lowest in control variant V1 (41.80 g), which was not fertilized.

During the production year 2016/17, the average 1000-grain weight of triticale was 39.84 g high temperatures and drought were recorded at the time of grain pouring, which affected the reduction of the 1000-grain weight. The highest 1000-grain weight in the second growing season had the variants V4 and V5 (40.34 g) and V1 variant, which was not fertilized, had the lowest (38.00 g).

During the production year 2017/18, the average 1000-grain weight for all tested fertilization variants was 42.22 g. During the examined period, the highest average 1000-grain weight was obtained in the V5 variant (43.56 g), where a combination of NPK with lime and organic fertilizer was applied and the lowest in the control variant (41.00 g).

Table 4. The analysis of variance for 1000 grain weight in Kraljevo, Republic of Serbia

Effect	Df	Mean sq Effect	Mean sq Error	F	p-level
Year, (Y)	2. 72	85.84120	3.045067	28.19025	0.000 ^{**}
Fertilization, (F)	4. 70	15.69047	4.688076	3.3469	0.014 [*]
Year x Fertilization, (YxF)	14. 60	17.41394	2.452200	7.1014	0.000 ^{**}

^{**}non significant; ^{*}significant at 0.05; ^{**}significant at 0.01;

The influence of year and fertilization, as well as their interaction on the 1000-grain weight of winter triticale, during three production years is shown in Table 4. The influence of production year on the 1000-grain weight of tested winter triticale was highly significant ($F_{\text{exp}} = 28.190^{**}$). During all investigated growing seasons, the 1000-grain weight of triticale was higher in all variants of fertilization compared to the control that was not fertilized, which was confirmed by the analysis of variance where fertilization showed a significant effect on 1000-grain weight ($F_{\text{exp}} = 3.347^*$). The interaction of year and fertilization was highly significant in affecting the 1000-grain weight of triticale ($F_{\text{exp}} = 7.101^{**}$).

Test weight

The average values of test weight with different variants of fertilization during three growing seasons are shown in Table 5.

Table 5. Test weight of winter triticale in Kraljevo, Republic of Serbia

Fertilization	Years						Average	
	2015/16		2016/17		2017/18			
	\bar{x}	S	\bar{x}	S	\bar{x}	S	\bar{x}	S
V1	73.37	1.534	67.84	2.037	72.57	1.480	71.26	2.977
V2	74.65	1.685	72.04	2.272	73.65	1.068	73.45	1.962
V3	74.53	0.576	70.71	1.921	73.13	1.230	72.79	2.061
V4	75.05	1.020	71.92	1.249	74.25	1.281	73.74	1.761
V5	76.25	1.225	73.01	1.071	74.65	1.225	74.64	1.749
Average	74.77	1.497	71.10	2.439	73.65	1.383	73.17	2.379

The average value of test weight during three growing seasons included in the research was 73.17 kg hl⁻¹. During the investigated period, the highest average test weight of triticale was achieved in variant V5 (74.64 kg hl⁻¹), which was fertilized with mineral NPK fertilizer in combination with lime and manure.

During the production year 2015/16, the average value of test weight for all tested fertilization variants was 74.77 kg hl⁻¹. During the first year, the highest average test weight was achieved in variant V5 (76.25 kg hl⁻¹), which was fertilized with NPK, lime and manure, and the lowest in V1 variant (73.37 kg hl⁻¹). The average value of test weight of winter triticale for all tested fertilization variants during the second year 2016/17 was 71.10 kg hl⁻¹. During the second production year, the highest average test weight was achieved in variant V5 (73.01 kg hl⁻¹) and manure and the lowest in the control variant which was not fertilized (67.84 kg hl⁻¹).

During the production year 2017/18, the average test weight of winter triticale for all tested fertilization variants was 73.65 kg hl⁻¹. During the examined period, the highest average test weight of triticale was obtained in the variants V4 and V5 (74.25 kg hl⁻¹; 74.65 kg hl⁻¹) and the lowest in the control variant (72.57 kg hl⁻¹).

Table 6. The analysis of variance for test weight in Kraljevo, Republic of Serbia

Effect	Df	Mean sqr Effect	Mean sqr Error	F	p-level
Year, (Y)	2. 72	88.23363	3.367217	26.20373	0.000**
Fertilization, (F)	4. 70	23.79355	4.624752	5.1448	0.001**
Year x Fertilization, (YxF)	14. 60	20.84813	2.117217	9.8470	0.000**

^{ns}non significant; ^{*}significant at 0.05; ^{**}significant at 0.01;

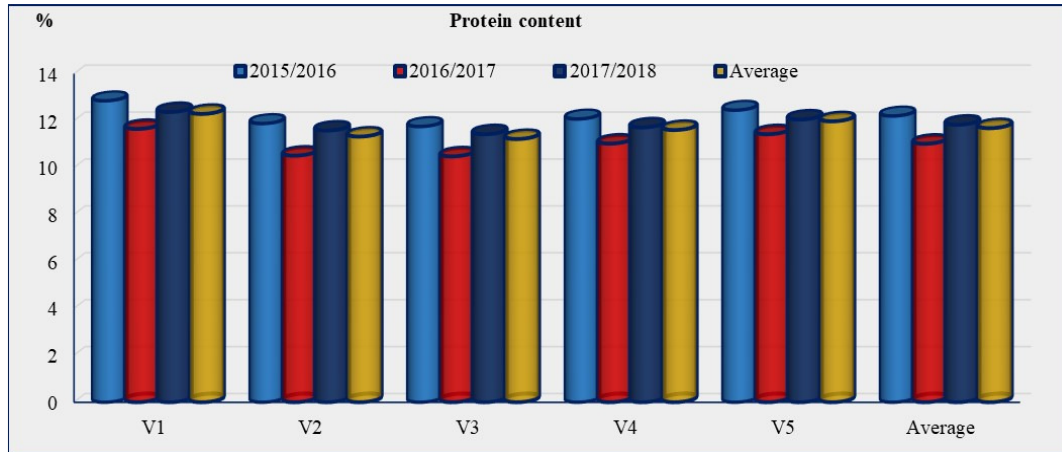
The obtained data on the test weight showed highly significant difference between the growing seasons, where on average for the examined years the highest test weight was produced in production year 2015/16 (Table 6). Based on the variance analysis data, highly significant influence of fertilization on test weight can be concluded ($F_{exp} = 5.145^{**}$). The interaction between the examined vegetation seasons and fertilization variants during the research had a highly significant effect on the test weight of triticale ($F_{exp} = 9.847^{**}$).

Protein content

In regard to protein content, with different variants of fertilization during the three growing seasons (Table 7), the average protein content of triticale in the experiment was 11.682%. The highest average protein content of triticale grains, during the investigated period, was achieved in variant V5 (11.975%), which was fertilized with mineral NPK fertilizer in combination with lime and manure, Table 7, Figure 5.

Table 7. Protein content of winter triticale in Kraljevo, Republic of Serbia

Fertilization	Years						Average	
	2015/16		2016/17		2017/18		\bar{x}	S
	\bar{x}	S	\bar{x}	S	\bar{x}	S		
V1	12.850	0.864	11.650	0.603	12.370	0.529	12.290	0.811
V2	11.880	0.382	10.510	0.551	11.580	0.284	11.323	0.722
V3	11.754	0.245	10.480	0.520	11.424	0.614	11.219	0.717
V4	12.074	0.553	11.024	0.691	11.714	0.458	11.604	0.698
V5	12.450	0.496	11.424	0.499	12.050	1.107	11.975	0.826
Average	12.202	0.646	11.018	0.713	11.828	0.695	11.682	0.840

**Figure 5.** Effect of fertilization on protein content of winter triticale in Kraljevo, Rep. of Serbia

The average value of protein content during the first growing seasons of research was 12.202%. During the production year 2015/16, the highest average protein content of triticale was achieved in variant V1 (12.850%), which was not fertilized and variant V5 (12.450%), which was fertilized with mineral NPK fertilizer in combination with lime and manure.

During the second growing season, the average protein content of triticale grains was 11.018%. The highest protein content in the production year 2016/17 had the control variant which was not fertilized (11.650%) and variant V5 (11.424%), which was fertilized with mineral NPK fertilizer, lime and manure. During the production year 2017/18, the average protein content of triticale grains was 11.828%. The highest protein content in the growing season 2017/18 had the variants V1 (12.370%) and V5 (12.050%).

The influence of year and fertilization, as well as their interaction on the protein content of winter triticale, during three growing seasons is shown in Table 8.

Table 8. The analysis of variance for protein content in Kraljevo, Republic of Serbia

Effect	Df	Mean sqr Effect	Mean sqr Error	F	p-level
Year, (Y)	2. 72	9.15763	0.470098	19.48027	0.000**
Fertilization, (F)	4. 70	3.01539	0.572868	5.2637	0.001**
Year x Fertilization, (YxF)	14. 60	2.19921	0.356222	6.1737	0.000**

^{ns}non significant; ^{*}significant at 0.05; ^{**}significant at 0.01;

The influence of production year on the protein content of triticale was highly significant ($F_{exp} = 19.480^{**}$). During all investigated periods, the analysis of variance showed a highly significant effect of

fertilization on protein content ($F_{exp} = 5.264^{**}$). The interaction of year and fertilization was highly significant in affecting the protein content of triticale grains ($F_{exp} = 6.174^{**}$, Table 8).

Correlation analysis of the studied oat traits

Correlation coefficients based on all tested traits of triticale grains during three growing seasons (2015/16, 2016/17 and 2017/18) had positive and negative values in Table 9. During 2015/16, highly significant positive dependencies were found between grain yield with 1000-grain weight ($r = 0.629^{**}$) and test weight ($r = 0.560^{**}$) and significant positive dependencies were found between 1000-grain weight and test weight ($r = 0.479^*$, Table 9).

Negative dependencies were found between protein content with grain yield ($r = -0.297$), 1000-grain weight ($r = -0.059$) and test weight ($r = -0.053$). During 2016/17, positive highly significant dependencies were found between grain yield with 1000-grain weight ($r = 0.597^{**}$) and test weight ($r = 0.660^{**}$) and positive significant dependencies were found between 1000-grain weight and test weight ($r = 0.408^*$, Table 9). Negative dependencies were found between protein content with grain yield ($r = -0.339$), 1000-grain weight ($r = -0.266$) and test weight ($r = -0.255$).

During 2017/18, positive significant dependence was determined between grain yield and test weight ($r = 0.434^*$). Positive and negative dependencies were found between grain yield with 1000-grain weight ($r = 0.334$) and protein content ($r = -0.334$). Positive dependencies were found between 1000-grain weight with test weight ($r = 0.355$) and protein content ($r = 0.025$) in Table 9.

Correlation coefficients based on all tested traits of triticale grains in different fertilizer variants had positive and negative values in Table 10.

Positive highly significant correlation coefficients, in the variant without fertilization (V1), were found between 1000-grain weight and test weight ($r = 0.709^{**}$) and positive significant dependencies were found between test weight with grain yield ($r=0.517^*$) and protein content ($r = 0.592^*$) in Table 10.

Table 9. Correlation coefficients by studied environments in winter triticale

Traits	GY	TGW	TW	PC
2015/16				
GY	1.000	0.629 ^{**}	0.560 ^{**}	-0.297
TGW		1.000	0.479 [*]	-0.059
TW			1.000	-0.053
PC				1.000
2016/17				
GY	1.000	0.597 ^{**}	0.660 ^{**}	-0.339
TGW		1.000	0.408 [*]	-0.266
TW			1.000	-0.255
PC				1.000
2017/18				
GY	1.000	0.334	0.434 [*]	-0.334
TGW		1.000	0.355	0.025
TW			1.000	0.334
PC				1.000

¹GY-Grain yield (t ha⁻¹), TGW-1000 grain weight (g), TW-Test weight (kg hl⁻¹), PC-Protein content (%);

²*significant at 0.05; **significant at 0.01;

Table 10. Correlation coefficients for the traits analysed across the fertilization variants

Traits	GY	TGW	TW	PC
V1-control				
GY	1.000	0.506	0.517 [*]	0.392
TGW		1.000	0.709 ^{**}	0.467
TW			1.000	0.592 [*]
PC				1.000
V2- NPK				
GY	1.000	0.272	0.111	0.441
TGW		1.000	0.220	0.500
TW			1.000	0.478
PC				1.000
V3- NPK in combination with lime				
GY	1.000	0.516 [*]	0.359	0.453
TGW		1.000	0.588 [*]	0.573 [*]
TW			1.000	0.719 ^{**}
PC				1.000
V4-NPK in combination with manure				
GY	1.000	0.695 ^{**}	0.516 [*]	0.566 [*]
TGW		1.000	0.653 ^{**}	0.648 ^{**}
TW			1.000	0.452
PC				1.000
V5-NPK in combination with lime and manure				
GY	1.000	0.640 [*]	0.648 ^{**}	0.332
TGW		1.000	0.698 ^{**}	0.409
TW			1.000	0.545 [*]
PC				1.000

¹ GY-Grain yield, TGW-1000-grain weight, TW-Test weight, PC-Protein content

² V1-control, V2-N₁₂₀P₈₀K₆₀, V3-N₁₂₀P₈₀K₆₀ + 5 t ha⁻¹ lime, V4-N₁₂₀P₈₀K₆₀ + 30 t ha⁻¹ manure and V5-N₁₂₀P₈₀K₆₀ + 5 t ha⁻¹ lime + 30 t ha⁻¹ manure

³ *significant at 0.05; **significant at 0.01;

Positive correlation coefficients, in the fertilizer variant with NPK (V2), were found between grain yield with 1000-grain weight ($r = 0.272$), test weight ($r = 0.011$) and protein content ($r = 0.441$) and positive dependencies were found between 1000-grain weight with test weight ($r = 0.220$) and protein content ($r = 0.500$) in Table 10.

In the NPK and lime variant (V3), highly significant positive correlation coefficients, were found between test weight and protein content ($r = 0.719^{**}$) and positive significant dependencies were found between grain yield and 1000-grain weight ($r = 0.516^{*}$) and between 1000-grain weight with test weight ($r = 0.588^{*}$) and protein content ($r = 0.573^{*}$) in Table 10.

In the NPK and manure variant (V4), highly significant positive correlation coefficients, were found between grain yield and 1000-grain weight ($r = 0.695^{**}$), between 1000-grain weight with test weight ($r = 0.653^{**}$) and protein content ($r = 0.648^{**}$) and positive significant dependencies were found between grain yield with test weight ($r = 0.516^{*}$) and protein content ($r = 0.566^{*}$) in Table 10.

Positive highly significant correlation coefficients, in the NPK, lime and manure variant (V5), were found between test weight with grain yield ($r = 0.648^{**}$) and 1000-grain weight ($r = 0.698^{**}$) and positive significant dependencies were found between grain yield and 1000-grain weight ($r = 0.640^{*}$) and between test weight and protein content ($r = 0.545^{*}$) in Table 10.

Discussion

Meteorological conditions

Unfavourable weather conditions (high and low temperatures, high amounts of precipitation with stormy and strong winds, drought, acidic and salty soils) have a strong negative impact on the production of triticale and represent a great risk, because they limit the manifestation of the maximum genetic potential of the cultivated crop (Fraš *et al.*, 2016; Dubis *et al.*, 2017; Kizilgeci and Yildirim, 2017; Bielski *et al.*, 2020; Dražić *et al.*, 2021; Kosev *et al.*, 2022; Milunović *et al.*, 2022; Lakić *et al.*, 2022). Due to climate change, Laidig *et al.* (2022) point out that increased efforts of breeders are needed to create new lines and varieties of triticale that would be tolerant to diseases and lodging. In addition to the new lines and varieties of triticale, the grain yield is greatly influenced by the fertilization system, which should be harmonized with climatic and soil conditions and variety requirements (Biberdžić *et al.*, 2017; Gerdzhikova *et al.*, 2017; Kirchev and Georgieva, 2017; Madić *et al.*, 2018; Bielski *et al.*, 2020; Derejko *et al.*, 2020; Petrović *et al.*, 2022; Popović *et al.*, 2020a; 2020b; 2022).

In the growing season 2017, during the budding period of cultivated triticale plants in March, due to insufficient moisture in the soil, the nitrogen fertilizer did not change into easily accessible forms and was not used by the plants, which resulted in a yield decrease. Based on the fact that the amount of precipitation and temperature in the period March-June are very important for the development of winter cereals, the first year of research 2015/16 can be characterized as more favourable in terms of the distribution and amount of precipitation compared to the growing season 2016/17 and production year 2017/18. During the largest part of the production year 2015/16, favourable weather conditions resulted in a very high yield and quality of triticale grains. Terzić *et al.* (2018) and Rajičić *et al.* (2020) state that the increase in strong winds and heavy rains in the future will call into question the stability of yields not only for triticale but also for other cereals.

Grain yield

The yield of winter triticale in 2015/16 is very good and is higher by 20% compared to the 2016/17 vegetation year and by about 6% compared to the 2017/18 production year. Losert *et al.* (2017) observed significant progress in triticale breeding and a significant rate of increase in triticale grain yield of 53 kg ha⁻¹ or 0.67% per year. Yields of winter triticale in research conducted by Bielski *et al.* (2020) significantly varied during the three-year experiment and ranged from an average of 4.56 t ha⁻¹ in 2013, 4.62 t ha⁻¹ in 2014 and 3.82 t ha⁻¹ in 2015. The effect of the year on grain yield in the winter triticale was not significant, which is in accordance with the results of Lalević *et al.* (2022). A significant influence of the growing season on grain yield in winter triticale was established by Dogan *et al.* (2011), Ivanova and Tsenov (2014), Janušauskaite (2014) and Kirchev and Georgieva (2017), in wheat by Kadar *et al.* (2018) and Popović *et al.* (2020) and for oats by Jelić *et al.* (2013) and Ugrenović *et al.* (2021). The analysis of variance in the research by Rajičić *et al.* (2020a) shows that the effect of year on grain yield is highly significant. Đekić *et al.* (2014) indicate that the highly significant effects of the year are also manifested as an increase in the winter triticale grain yield. Ivanova and Tsenov (2014) point out that the vegetation seasons influence considerably the formation of their yield (46%) and that mineral fertilization had the greatest effect on yield formation in new varieties of triticale with 17%.

In the production year 2015/16, the highest average yield of triticale for all analyzed variants of fertilization recorded was significantly higher than the yield recorded during the production 2016/17. By analysis of variance, individual influences of fertilization on grain yield were significant at the 0.01 level. The highly significant effect of fertilization on grain yield of winter triticale is established by Đekić *et al.* (2014), Ivanova and Tsenov (2014), Terzić *et al.* (2018) and Lalević *et al.* (2022). Bielski *et al.* (2020) indicate that the highest grain yield of triticale during the three-year experiment is obtained in the treatment with 120 kg ha⁻¹ nitrogen fertilization (4.80 t ha⁻¹) and the lowest in the control treatment without nitrogen fertilization (3.17 t ha⁻¹). The high effect of combined application of NPK fertilizers on triticale grain yield is established by Rajičić *et al.* (2020). On average for all fertilization variants, during a three-year trial, the highest yield of triticale is

reported by Rajičić *et al.* (2020) on the variant with combined application of NPK (80 kg ha⁻¹ N, 100 kg ha⁻¹ P₂O₅ and 60 kg ha⁻¹ K₂O). Lalević *et al.* (2022) point out that for all tested variants of fertilization in both years of testing the highest average yield is observed for the variant of 120 kg ha⁻¹ nitrogen fertilization (6.60 t ha⁻¹ in the first and 6.37 t ha⁻¹ in the second year). The high impact of combined nutrition of NPK, lime and manure on grain yield on acidic soils is determined by Biberdžić *et al.* (2017), Rajičić *et al.* (2020) and Babić *et al.* (2023), which is consistent with our research. The positive effect during the complete application of fertilizers is the result of a lower pH value of the soil, as well as a low content of available phosphorus and potassium in vertisol type soil. The results of the research by Rajičić *et al.* (2020) and Babić *et al.* (2023), indicate the importance of rational introduction of adequate amounts of mineral fertilizers in order to produce triticale as profitably as possible.

The results of Lalević and Biberdžić (2016) show that the use of nitrogen has a positive effect on the yield and yield components of winter triticale in all fertilization variants and in all varieties. A nitrogen fertilizer rate of 120 kg N ha⁻¹ combined with two foliar fungicide treatments exerted the most beneficial influence on the yield and disease resistance of semi-dwarf winter triticale (Dubis *et al.*, 2017). A nitrogen fertilizer rate of 120 kg N ha⁻¹ exerted the most beneficial influence on the yield of winter triticale (Lalević and Biberdžić, 2016; Terzić *et al.*, 2018; Bielski *et al.*, 2020; Rajičić *et al.*, 2020; Lalević *et al.*, 2022). The combined application of mineral NPK fertilizer with lime and manure resulted in a significant increase in fertility of the studied pseudogley soil, which was confirmed by a significant increase in the yield of triticale with this particular fertilizer variant (NPK + lime + manure). The research results obtained by Rajičić *et al.* (2020) show that the highest grain yield (3.802 kg ha⁻¹) is obtained with the oats in the combined application of NPK, lime and manure.

Thousand grain weight

The average 1000-grain weight of triticale for all investigated growing seasons and fertilization variants during the research period trial was 41.85 g. The highest 1000-grain weight in the first growing season (2015/16) was 45.36 g, in the second growing season (2016/17) 40.34 g and in the production year (2017/18) it was 43.56 g. In a study carried out in Erzurum with 14 triticale genotypes for two years, Kucukozdemir *et al.* (2018) established the 1000-grain weights of 25.50-33.50 g, 37.50-49.20 g. They established that the precipitation especially in June and July 2016 increased the 1000-grain weight and yields however protein ratios decreased. According to the two-year average 1000-grain weight of triticale cultivars changed from 38.85-45.12 g (Sirat *et al.*, 2022). The effect of the year on 1000-grain weight in winter triticale is significant at the 0.01 levels which is in accordance with the results of Đekić *et al.* (2014) and Ivanova and Tsenov (2014). Growing season shows a significant effect on 1000-grain weight for winter triticale as established in a study by Terzić *et al.* (2018), Rajičić *et al.* (2020) and Sirat *et al.* (2022) and for oats in a study by Jelić *et al.* (2013).

By analysis of variance, individual influences of fertilization on the 1000-grain weight were significant at the 0.05 level. The highly significant effect of fertilization on 1000-grain weight of winter triticale has been established by Terzić *et al.* (2018) and Lalević *et al.* (2022). By analysis of variance, the effect of interaction of years and fertilization on 1000-grain weight were significant at the 0.01 level, which is in accordance with the results of Ivanova and Tsenov (2014), Terzić *et al.* (2018) and Lalević *et al.* (2022). Ivanova and Tsenov (2014) point out that the meteorological conditions (vegetation seasons) influence the very low degree of the 1000-grain weight (22%) and that mineral fertilization has the greatest effect on 1000-grain weight formation in new varieties of triticale with 13%. Terzić *et al.* (2018) in a three-year study of the impact of the application of mineral nutrients on the 1000-grain weight, in winter triticale, report the highest values in the treatment fertilized with 120 kg ha⁻¹ N and 60 kg ha⁻¹ P₂O₅ in 2009 (48.14 g), in the treatment fertilized with 120 kg ha⁻¹ N, 60 kg ha⁻¹ P₂O₅ and 60 kg ha⁻¹ K₂O in 2010 (44.00 g) and 2011 (43.64 g). Bielski *et al.* (2020) have established a significant influence of nitrogen fertilization on the 1000-grain weight. The highest values of the 1000-grain weight are obtained in the variant with the lowest dose of nitrogen 40 kg ha⁻¹ (40.1 g) and the lowest

in the variant without nitrogen fertilization (36.4 g). Increasing the dose of nitrogen for fertilization to 120 kg ha⁻¹ has led to a significant decrease in the 1000-grain weight (38.8 g). During the two-year research Lalević *et al.* (2022) have showed the existence of significant differences in the values of the 1000-grain weight of the tested fertilization variants. Average two-year values for all fertilization variants show that the average 1000-grain weight is the highest in the variant where nitrogen is used in the amount of 120 kg ha⁻¹ and in the first year of the research it is 44.3 g, and in the second year it is 43.2 g. The application of even the lowest amount of nitrogen in the cultivation of triticale significantly affects the increase in the 1000-grain weight (Oral, 2018; Bielski *et al.*, 2020).

Test weight

The average test weight of triticale for all investigated growing seasons and fertilization variants during the research period trial was 74.64 kg hl⁻¹. The highest test weight in the production year 2015/16 was 76.25 kg hl⁻¹, in the growing season 2016/17 it was 73.01 kg hl⁻¹ and in the second year 74.65 kg hl⁻¹. In a study by Kucukozdemir *et al.* (2018) the test weights of the 14 triticale genotypes for two years according to years are reported as 75.20-80.00 kg hl⁻¹, 73.20-79.60 kg hl⁻¹, respectively. Sirat *et al.* (2022) state that the test weight of triticale varies significantly depending on the growing season and the test weight of cultivars changes from 74.68 kg hl⁻¹ to 77.21 kg hl⁻¹. From the data of Lalević *et al.* (2022), the highest test weight achieved was 69.9 kg hl⁻¹ in the first year (2017/18) and 66.4 kg hl⁻¹ in the second year (2018/19). The effect of the year on test weight in the winter triticale was significant at the 0.01 level, which is in accordance with the results of Đekić *et al.* (2014) and Rajičić *et al.* (2020). The environment has a significant effect on test weight for winter triticale in a study by Terzic *et al.* (2018) and Sirat *et al.* (2022) and for oat in study by Jelić *et al.* (2013).

By analysis of variance, individual influences of fertilization on test weight were significant at the 0.01 level. Ivanova and Tsenov (2014) point out that the vegetation seasons influenced to the strongest effect on the test weight of the new varieties of triticale (80%), but mineral fertilization had the greatest effect on yield formation in new varieties was insignificant on the test weight of 3.5%. By analysis of variance, the effects interaction of years and fertilization on test weight were significant at the 0.01 level, which is in accordance with the results of Lalević *et al.* (2022). Rajičić *et al.* (2020a), point out that the test weight during 2013 year significantly varied across treatments from 68.03 kg hl⁻¹ g in treatment N₈₀P₁₀₀K₆₀ to 71.89 kg hl⁻¹ in treatments N₈₀P₆₀K₆₀. From the data of Lalević *et al.* (2022), it can be seen that the treatment with 120 kg ha⁻¹ N, in both years of the research, achieved the highest test weight was 72.4 kg hl⁻¹ and 68.7 kg hl⁻¹ in the treatment N₁₂₀P₁₀₀K₈₀.

Protein content

According to literature data, there are significant variations regarding the chemical composition and nutritional properties of triticale, which is a consequence of the presence of a large number of genotypes with very different properties (Fras *et al.*, 2016; Gerdzhikova *et al.*, 2017; Perišić *et al.*, 2019; Sirat *et al.*, 2022).

The highest average protein content of winter triticale grains during research was 11.975%, in 2015/16 it amounted to 12.450%, while the lowest protein content 11.424% was found in 2016/17. The results obtained from the study by Djekic *et al.* (2011), confirm that newer varieties and lines of winter triticale have a lower protein content in the grain compared to lamb. Examining eight Polish winter cultivars of hexaploid triticale Fras *et al.* (2016) find that the protein content in the grain of the examined varieties ranges from 11.8% to 15.2%. In another study examining fourteen triticale genotypes for two years (Kucukozdemir *et al.*, 2018), the following grain protein ratios are reported, 13.83-15.20% and 11.28-13.27%, respectively. In a study conducted in experimental areas in Erzurum (Pasinler and Erzincan) under dry conditions, Kucukozdemir *et al.* (2019) examined two registered varieties and thirteen lines of triticale during 2017/18. They have found that the protein content in the grains of the tested triticale varieties and lines in the Pasinler area ranges from

9.0-13.9%, while in the Erzincan area the protein content ranges between 13.1-17.1%. The protein content in the grain of nine different varieties of triticale during a two-year study range from 10.36-12.69% (Sirat *et al.*, 2022). Camerlengo and Kiszonas (2023) point out that triticale has a high nutritional value due to the composition of proteins whose content in triticale grains ranges from 8.6% to 16.3%. The effect of the growing season on protein content in the winter triticale is significant at the 0.01 level. The growing season has a significant effect on protein content for winter triticale as established in a study by Djekic *et al.* (2014) and Sirat *et al.* (2022) and for oats in a study by Jelić *et al.* (2013). The highest protein content in triticale grains in the study had a variant without fertilization (12.290%) and the lowest variant with combined nutrition of NPK and lime (11.219%).

By analysis of variance, individual influences of fertilization on protein content in the winter triticale were significant at the 0.01 level, which is in accordance with the results of Lalević *et al.* (2022). Based on research of Gerdzhikova *et al.* (2017) and Flajšman *et al.* (2020) the use of mineral nitrogen reduces the protein content. Data obtained by Lalević *et al.* (2022), show that the treatment with 60 kg ha⁻¹ N, in both years of the research, achieves the highest protein content in triticale grains 14.15% in the first (2017/18) and 13.48% in the second year (2018/19). The protein content in the grain of the tested triticale cultivars in the study by Lalević *et al.* (2022) ranges from 11.08% in the cultivar Tango in the variant without fertilization in 2018/19 year to 14.64% in the cultivar Favorit in the variant using 100 kg N ha⁻¹ in the 2017/18 year of research. We have established significant differences in the protein content in different growing seasons and the different fertilization variants, indicating how much influence the years and fertilization can have on the protein content in triticale grains, which is in accordance with the results of Janušauskaitė (2014).

Correlations between the analysed traits

The study results indicated that the grain yield in all vegetation seasons was positive and highly significantly correlated with 1000-grain weight, except in the 2017/18 season, which is in line with the results of Terzić *et al.* (2018) and Rajičić *et al.* (2020) who also noticed in their experiments the appearance of a positive correlation between the mentioned quantities ($r = 0.79^{**}$ or $r = 0.65^{**}$). The positive and significant correlation with grain yield and 1000-grain weight of winter triticale in the three vegetation seasons ($r = 0.71^{*}$ in 2009/10, $r = 0.52^{*}$ in 2010/11 and $r = 0.54^{*}$ in 2011/12) has been established by Đekić *et al.* (2014). Using an association mapping approach Liu *et al.* (2017) has found a low phenotypic correlation between grain yield and biomass yield and concluded that selection based on markers for grain yield or biomass yield does not negatively affect 1000-grain weight. The negative and significant correlation with grain yield and 1000-grain weight of the tested genotypes of winter triticale has been established by Bielski *et al.* (2020).

The study results indicated that the grain yield in all fertilization variants was positive and significantly correlated with 1000-grain weight, except in the variants V2 with NPK and V3 with NPK and lime. Positive and significant correlations of grain yield and 1000-grain weight in fertilization have been established by Rajičić *et al.* (2020a). A negative and not-significant correlation between grain yield and 1000-grain weight of winter triticale has been established by Đekić *et al.* (2014) and Terzić *et al.* (2018).

The correlative dependence indicated that the grain yield in all vegetation seasons was positive and highly significantly correlated with test weight, except in the 2017/18 season where a significant correlation was established. A positive and significant correlation of grain yield and test weight of winter triticale in vegetation season has been established by Đekić *et al.* (2014) and Terzić *et al.* (2018) ($r = 0.44^{*}$ or $r = 0.49^{*}$). A negative and highly significant correlation between grain yield and test weight of winter triticale ($r = -0.64^{**}$) has been established by Rajičić *et al.* (2020a).

The study results indicated that the grain yield in all fertilization variants was positive and significantly correlated with test weight, except in the V3 variant with NPK and lime where a non-significant correlation was established. Negative and significant correlation between the yields of oats and test weight are established

by Jelic *et al.* (2013), but positive and non-significant correlations of grain yield and test weight are established by Đekić *et al.* (2014), Terzić *et al.* (2018) and Rajičić *et al.* (2020).

The study results indicated that the 1000-grain weight in all vegetation seasons was positive and significantly correlated with test weight, except in the 2017/18 season where a non-significant correlation was established. A positive correlation of 1000-grain weight and test weight of winter triticale in vegetation season has been established by Đekić *et al.* (2014) and Terzić *et al.* (2018). A negative correlation between 1000-grain weight and test weight of winter triticale has been established by Rajičić *et al.* (2020).

Correlations between grain yield and protein content of winter triticale show a negative value in all investigated vegetation seasons. Based on the results of the correlation coefficients, Lalević *et al.* (2020) point out that the yield of triticale is negatively correlated to protein content ($r = -0.92$), which is in agreement with our study. Also, Salehi and Arzani (2013) report in their experiments the appearance of a negative correlation between the grain yield and protein content ($r = -0.72$). Rajičić *et al.* (2020) have established negative and highly significant dependencies between yield and protein content in the three vegetation seasons ($r = -0.766^{**}$ in 2015 and $r = -0.766^{**}$ in 2017) and negative and significant dependencies between yield and protein content ($r = -0.773^{*}$ in 2016). Analyzing the interdependence of oat grain yield and protein content of the grain, with different fertilization variants, Jelic *et al.* (2013) state that their correlation is positive with NPK fertilization ($r = 0.63^{*}$) and with humification with calcification ($r = 0.55$).

Conclusions

The results of examining the influence of growing seasons and different fertilizer variants on yield, yield components and protein content in triticale grain during the three-production year period indicate the existence of differences in the achieved results between the examined years and between the fertilization variants. During the largest part of the production year 2015/16, favourable weather conditions resulted in a very high yield and quality of triticale grains. During the same year, the highest average yield of triticale for all analyzed variants of fertilization was achieved which is significantly higher than the yield recorded during the production 2016/17. A high effect on yield, 1000-grain weight, test weight and protein content of applied fertilizers was achieved by the combined use of NPK, lime and manure (120 kg N ha^{-1} , $80 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$, $60 \text{ kg K}_2\text{O ha}^{-1} + 5.0 \text{ t ha}^{-1} \text{ lime} + 20 \text{ t ha}^{-1} \text{ manure}$). The impact of climatic factors on the yield components and quality of triticale grains was highly significant. The highly significant effect of fertilization on yield, yield components and protein content of triticale grains were determined. The integrated application of certain pedo-ameliorative measures (calcification and humification) and mineral NPK fertilizers in combination with varieties better adapted to low soil pH values is the optimal solution for increasing yield stability and the quality of produced triticale grain on acidic pseudogleys.

Authors' Contributions

All authors have participated in this research. VR has designed, supervised and written the paper; VR, NĐ, JS, MG and DT have participated in the experimentation and sample collection; Conceptualization: VP, VR, VP, MB and VB have analysed the data obtained; VR and DT have overseen the project and revised the manuscript. All authors have read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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