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## **ANALYSIS OF GENOTYPE-BY-YEAR INTERACTION FOR *Secale cereale* L. PRODUCTIVE TRAITS AND CIRCULAR ECONOMY**

### **SUMMARY**

The circular economy offers a new product-waste-product model, in this case obtaining biofuels from rye biomass. The circular economy introduces a new product design, which will enable its functionality for a longer period of use. *Secale cereale* L. is an economically important crop for food, feed and bioenergy. The objective of this study was to estimate productivity of rye genotypes and the possibility of obtaining biogas from rye biomass during two growing seasons, 2019-2020. The aim of this study was to examine the influence of year and genotype on rye productivity parameters, biogas, methane yield, methane proportion, and the possibility of using rye as an alternative fuel in Serbia. The influence of the year and genotypes on the parameters of rye productivity, biogas and methane yield, methane content and the possibility of using rye as an alternative fuel in Serbia was investigated. Genotype and year × genotype interaction had a statistically significant effect on biogas yield, methane yield and methane content in the studied rye genotypes. Genotype G1 had the mean of green biomass yield (25.73 t ha<sup>-1</sup>) significantly higher compared to genotype G2 (23.75 t ha<sup>-1</sup>) in both years of experiment. Green biomass yield (24.11 t ha<sup>-1</sup>) was better in 2019 compared to 2020. Biogas yield varied from 260.57 m<sup>3</sup> ha<sup>-1</sup> (genotype G1) to 214.58 m<sup>3</sup> ha<sup>-1</sup> (genotype G2). Biogas yield were better in 2019

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(237.85 m<sup>3</sup> ha<sup>-1</sup>) compared to 2020 (237.30 m<sup>3</sup> ha<sup>-1</sup>). A positive statistically highly significant correlation was attained between the green biomass yield and the length of the spikes (0.82\*\*), green biomass yield and biogas yield (0.93\*\*), green biomass yield and methane content (0.90\*\*).

**Keywords:** rye; biomass, biogas yield, G×Y interaction; circular economy.

## INTRODUCTION

After wheat, rye is the second most important raw material for bread and bakery products, and it is one of the most excellent sources of dietary fibres and bioactive compounds. Rye is utilised in more other food products as well, such as breakfast cereals, porridges, pasta, snack products, etc. Recent scientific research is focused on studying the possible health benefits and the potential of rye in the development of novel food products but also the possibility of using it for energy purposes. Rye (*Secale cereale* L.) is a small grain cereal perfectly adapted to different agroecological conditions, so that it has a very large area of distribution. Areas under rye are significantly reducing in the world every year. In 2018, rye was grown on 4,403,020 ha in the world and on about 5,000 ha in Serbia. The main product is grain, which is mostly used for bread and bakery products and in the industry for the production of alcoholic beverages (Jordanovska *et al.*, 2018). Rye is main source of starch and energy. Rye grain contains numerous nutritional components such as proteins, fats, vitamins (B complex), dietary fibre and phytochemicals. Improving drought tolerance has always been an important objective in many crop improvement programs and is becoming more important as one way of adapting crops to climate changes (Belitz *et al.*, 2009; Lakew *et al.*, 2018). Nowadays, a larger number of hybrids are grown worldwide since they are more tolerant to drought in all phenophases (Ikanović *et al.*, 2013). In the past decades, many countries, under strong pressure to improve energy security from the aspect of environmental protection, but also to reduce dependence on imports, have begun to develop programs for the production of alternative biofuels (methane, ethanol and biodiesel) from plant products. Initially, the main crop products grain and fodder biomass were used for this purpose, while more recently systems for the use of waste of biological origin have been developed, with special emphasis on secondary-alternative-crop products and forest products (Janković *et al.*, 2019).

Circular economy is an "instrument" for the realization of sustainable development goals and implies long-term investment in raw material and energy efficiency, with reduction of harmful emissions, replacement of fossil fuels with renewable sources and production and trade in sustainable products, thus closing the circle "product-waste-product". Thanks to the development of new technologies for processing bio-waste into energy sources, the growth rate of the use of alternative fuels is growing significantly. According to estimates by energy experts, it is about 15% per year in highly developed countries. During the 21<sup>st</sup> century, population growth will be a big problem in finding solutions to provide the necessary amounts of food, but also energy, since the reserves of basic energy

sources, which are fossil fuels, are limited. According to the forecasts of experts in these fields, food and energy consumption will double by 2050 (Popović *et al.*, 2020a; Rakašćan *et al.*, 2021).

By improving the technological process of obtaining biofuels from secondary products, energy sources would be obtained with much wider application. The advantage of these energy sources is the fact that they come from renewable sources, which significantly reduces the dependence on the import of fossil fuels, which a large number of countries do not have. Another positive effect would be significantly lower emissions of harmful gases into the atmosphere. By burning biofuels, all the carbon dioxide that goes into the atmosphere would be used by plants for photosynthesis processes during the year, and at the same time they release oxygen. The amounts of other harmful gases released by burning of these alternative fuels are also much lower than of fossil fuels. The combustion of these alternative fuels are also far less than from fossil fuels. Biomass of secondary products, which would be used to produce biofuels, is one of the ways in which countries could meet their obligations under the Kyoto Protocol on Climate Change, because, as a whole, they would reduce greenhouse gas emissions and the greenhouse effect, as a fundamental factor in global temperature rise (Ikanović *et al.*, 2013; 2018).

During the anaerobic digestion process, biogas is produced together with a valuable residual stream known as the digestate. Therefore, increasing demand for biogas-based energy generation will generate a significant increase in the annual volumes of digestate generated. Recycling the digestate back to soil and therefore valuable nutrients such as nitrogen, potassium, phosphorus and organic carbon for plants is the circular economy concept case (Provenzano *et al.*, 2018). Anaerobic digestion is a good example of a closed loop process as the biogas is produced from the volatile matter fraction of various biodegradable feedstock streams such as animal slurry, manure or agricultural waste biomasses and the valuable nutrients available in the digestate are recycled back to the soil. Economic and environmental sustainability is challenged by two major factors: by the distance and feedstock quantity used for biogas production and the amount of digestate generated during the anaerobic digestion process in each biogas power plant. The leading strategy for a circular economy-based digestate management approach is still in its immature phase (Peng and Pivato, 2017).

In addition to economic problems, there are also problems of environmental protection, because the increasing use of fossil fuels significantly increases the amount of harmful gases in the atmosphere. The consequence of increasing the concentration of these gases in the atmosphere (especially carbon dioxide) affects climate change, which is manifested by global warming of the planet due to the greenhouse effect.

According to the results of the research, which are stated by British authors, if the straw of cereals, grown only in the area of the eastern part of the Midlands, were used to obtain biofuels, the amount of obtained energy would cover about 1.5% of British consumption. However, the views of local farmers

are clear and they insist that these secondary products be returned to the soil by ploughing or as manure, which has a far greater importance on soil fertility and further plant production. Finding the optimal solution for the use of grain straw should be the subject of further research (Collins *et al.*, 2014).

Rye has relatively drought tolerant compared to other cereal crops. Rye quality is very different from year to year (Đekić *et al.*, 2017; Đurić *et al.*, 2021). Examining the influence of genotype and environment on grain and quality traits, in a study with 19 different hybrid and population varieties grown for different years, found that variation in grain yield and protein concentration was mainly due to genotypes, but that thousand grain mass and dietary fiber concentration was more strongly influenced by harvest year than by genotype (Jordanovska *et al.*, 2018).

Grain yield is a function of genotype, environment and genotype x environment interaction (GEI) (Kota *et al.*, 2013; Djuric *et al.*, 2018; Đekić *et al.*, 2018; Kartina *et al.*, 2019; Amzeri *et al.*, 2020; Luković *et al.*, 2020; Khadka *et al.*, 2020; Rajičić *et al.*, 2021). An understanding of environmental and genotypic causes of GEI is important at all stages of crop improvement as they have a bearing on parent selection, selection based on yield as well as cultivar adaptation. GEI studies thus provide a basis for selection of genotypes that are suitable for general or specific cultivation; they also provide information about the effect of environment on cultivar performance (Khan *et al.*, 2007). The presence of genotype by environment ( $G \times E$ ) interaction is a major concern to rye breeders, since large interactions can reduce gains from selection and complicate identification of superior cultivars.

The objectives of our study are: a) to evaluate the influence of genotypes and environment on variation of productivity traits, b) to investigate correlations between traits, c) to evaluate rye production in divergent years and assess the possibility of using rye as an energy crop and d) to point out the importance of the circular economy. This study suggests that the stability analysis may contribute to additional information on the performance of new rye selections prior to release for commercial cultivation and may increase the effectiveness of cultivar development programs.

## MATERIAL AND METHODS

The experiment was conducted in Ilandza, Serbia, for two consecutive years (2018/2019 and 2019/2020). The trials were conducted according to a randomized block system in three replications with genotypes: G1- Propower (KWS) and G2- NS Savo (Institute of Field and Vegetable Crops, Novi Sad). Elementary plots were 10 m<sup>2</sup>. The genotype G1 is energy while genotype G2 for the grain. During the experiment, the standard cultivation technology for rye production was applied. Preceding crop was soybean. At the pre-sowing preparation, NPK nutrients (350 kg ha<sup>-1</sup>) were introduced. Sowing was 10/21/2018 and 10/26/2019 with cereal seeder. During the vegetation period, three time mechanical crop care measures were applied. Mowing was performed

at waxy ripeness and then samples were taken, from each elementary plot, for the analysis of the morphological productive parameters. After the morphological parameters were measured, each of rye genotypes was separately cut and placed in trench silos, and covered for 40 days. After that the silage was placed in a fermenter and biogas was obtained from it.

Rye is sown until late September or early October. Rye tolerates temperatures very well from minus 25 degrees. It has a higher tolerance to diseases than wheat. For feeding new genotypes of rye 100-110 kg of nitrogen per hectare is needed, 60-80 kilograms of phosphorus and 40-60 kilograms of potassium. New generation genotypes achieve a yield of 7 to 8.5 tons per hectare. Rye is grown for grain and for silage but also for bioenergy. Silage is done in April, while the rye is still green. Rye then has a lot of protein quality livestock feed was obtained. Haylage yields are about 24,000 kilograms per hectare. Energy rye hybrids give higher biomass yield. The rye harvest for bioenergy is done around the middle of May (Glamočlija *et al.*, 2015; Lakić *et al.*, 2018). Rye by-products (biomass) is a good raw material for the production of alternative fuels.

Genotype G1 was selected for biogas production while genotype G2 was selected for grain and has high quality grains for various applications. Commonly in breeding for drought tolerance, grain yield is the basis for selection, but it is a complex, late-stage trait, affected by many factors aside from drought. For successful grain production, selection genotypes G2 is recommended, while for biogas production is recommended to the G1 genotype. If the goal of selection is to obtain a genotype for biogas production selection should focus on obtaining higher plants genotypes that is to obtain genotypes with higher biomass production.

#### ***Meteorological data***

Weather conditions have a significant influence on biomass production and plant yield (Lakić *et al.*, 2018; 2020; Terzić *et al.*, 2019; Popović *et al.*, 2020a; 2020b; Rajičić *et al.*, 2020; Ljubičić *et al.*, 2021). This experiment was conducted for two years in Ilandza (45° 10' 06" N; 20° 55' 06" E, 59 m above sea level), on a sandy chernozem soil, in the municipality of Alibunar, in the South Banat district of Serbia.

During the vegetation period, the two years were significantly different. The total amount of precipitation was 484.3 mm in 2018/2019 and 538.3 mm in 2019/2020 (Table 1).

The average temperatures were 9.5°C in 2018/2019 and 9.6°C in 2019/2020. The total precipitation in 2018/2019 was lower by 54 mm compared to 2019/2020 and by 40 mm compared to the long-term period (Tab. 1).

#### ***Soil Analysis***

The chemical analysis was performed of agrochemical characteristics of the soil in the municipality of Ilandza, on sandy chernozem soil, (Map 1a, 1b).

**Table 1.** Average temperature and total precipitation during the vegetation period in 2018/2019 and 2019/2020, Alibunar meteorological station

Parameter Year	Mounts								
	10.	11.	12.	1.	2.	3.	4.	5.	Average
<b>Mean monthly temperature</b>									
2018/2019	11.2	8.6	2.8	-0.2	3.2	9.5	11.0	17.2	9.5
2019/2020	11.5	9.0	2.0	0.3	3.1	8.5	11.9	18.0	9.6
Long term	12.6	7.5	2.0	0.8	2.6	7.6	13.0	18.0	9.6
<b>Monthly precipitation sums</b>									
2018/2019	36.2	41.6	33.2	47.2	20.3	32.6	67.2	90.0	484.3
2019/2020	48.3	52.4	62.1	48.3	39.2	52.0	72.1	93.4	538.3
Long term	48.8	52.0	45.8	51.0	55.2	55.8	48.9	82.3	524.0



(a)



(b)

**Map 1.** Locality of Ilandza on the map of Serbia (a); Municipality of Alibunar, South Banat district, Serbia (b).

Soil samples were collected at 2018. Composite soil sample (0–30 cm depth) was a combination of five subsamples (one sample from each corner and one from the center of a 10 m<sup>2</sup>). The following soil properties were measured according to standard methods: CaCO<sub>3</sub>, soil pH, total nitrogen (TN), available phosphorus (AP), available potassium (AK) (according to Bogdanović and Ubavić, 1995). Analysis of the results obtained have shown that pH in H<sub>2</sub>O is of weakly-alkaline (7.9) and pH in KCl was of neutral reaction (6.9), content of CaCO<sub>3</sub> was 2.1%, total nitrogen is 0.2% and very low content of available P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O (0.6 mg/100 g and 0.4 mg/100 g of soil).

### Biogas and methane production

Biogas from biomass is one of the possibilities of renewable energy production to reduce greenhouse gas emissions. The biogas yield (BGY) was determined by analysis of biomass in the laboratory of the Faculty of Engineering in Novi Sad, according to the method of VDI 4630 and was converted to  $\text{Nm}^3\text{t}^{-1}$  (Pham *et al.*, 2013). Energy crops are grown for the purpose of bioenergy production. Biomass now makes up the largest potential of agricultural residues, it is a versatile energy source that can substitute fossil energy in the energy sectors electricity. Biogas has an important role in the field of environmental conservation by mitigating global warming and conserving fossil fuel. The production of biogas from biomass or waste is one way to reduce both the consumption of crude oil and environmental pollution (Rakaščan *et al.*, 2021; Popović *et al.*, 2020a; Dražić *et al.*, 2021). The composition of biogas depends on the composition of the parent feedstock, its physical and chemical properties are similar to those of conventional diesel. Biogas is considered safe for the environment, showing insignificant contribution of carbon dioxide and particulate emissions (Zhu, 2018; Milanović *et al.*, 2020; Popović *et al.*, 2020a; Rakaščan *et al.*, 2021). Biogas is produced by the process of anaerobic digestion or fermentation. Anaerobic digestion is an established technology, we used it to treat a biomass - organic wastes. It is a biological process in which organic carbon, by oxides-reduction processes, is converted to the highest oxidation rate ( $\text{CH}_4$ ), Table 10. This process takes place in the absence of oxygen and is catalyzed by many microorganisms (Cakić and Stamenković, 2009).

**Table 2.** Biogas components produced in anaerobic biogas reactors.

Compound	Methane	Carbon dioxide	Nitrogen	Hydrogen	Hydrogen sulfide	Oxigen
Formula	$\text{CH}_4$	$\text{CO}_2$	$\text{N}_2$	$\text{H}_2$	$\text{H}_2\text{S}$	$\text{O}_2$
Percentage by volume, %	50-70	25-50	0-10	0-1	0.1-0.5	0-0.5

Source: [www.kolumbus.fi](http://www.kolumbus.fi).

Plants biomass can be used to produce briquettes and pellets, solid fuels suitable for use in smaller boiler plants, for example for heating residential buildings. It can also be used to produce liquid biofuels (ethanol) because it has high amounts of carbohydrates. The technological process of processing into ethanol is carried out in stages. In the first phase, the biomass is chopped and treated with sodium hydroxide to break down the lignin, then, it is hydrolyzing by ferments that break down the complex sugars to hexoses. During the fermentation process, the hexose sugars are converted into ethyl alcohol by the glucoamylase ferment, releasing carbon dioxide ( $\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2 \text{C}_2\text{H}_5\text{OH} + 2 \text{CO}_2$ ). In the distillation process, ethanol is separated from other by-products. Approximately 2 kg of glucose is required to obtain 1 kg of ethanol (Richards *et al.*, 1994; Milanović *et al.*, 2020). The estimation of G, E and  $G \times E$  interactions (or GEI) ensures valid recommendations of suitable varieties able to overcome the pressure due to variable occurring conditions. The determination of GEI factors

helps geneticists in their breeding programs to shift the selection toward varieties suited for wide environments or specific to certain niches. The production success was cultivar-dependent and the pedoclimatic conditions as essential factors in determining yield (Egea-Gilabert *et al.*, 2021).

The weekly methane production was calculated by multiplying the mass of individual substrate fed into the full-scale digesters on a basis with the properties of the different substrates determined in the laboratory. The obtained methane production of the substrates were added up supposing additivity without synergistic or inhibitory effects. The methane production was calculated as follows:

$$P = \sum Q_i \times TS_i \times VS_i \times BMP_i [\text{Nm}^3 \text{CH}_4 \text{ week}^{-1}]$$

where Q is the mass of substrate fed into the digesters per week [tons], TS is the total solids content of the substrate [%], VS is the volatile solids content of the substrate [%], and BMP is the biomethane potential of the substrate [ $\text{Nm}^3 \text{CH}_4 \text{ tVS}^{-1}$ ]. The data on the mass of substrates fed per week were provided by the operators of the two full-scale AD plants (Holliger *et al.*, 2017).

### **Statistical Analysis**

The experimental data obtained were analyzed by descriptive and analytical statistics, with the statistical package STATISTICA 12 for Windows (StatSoft). Testing the significance of the differences between the calculated mean values of the examined factors (Genotype and Years) was done by using a two-factor model of variance analysis. All significance ratings were derived from the LSD test for a significance level of 0.05% and 0.01%. The relative dependence between the tested parameters for rye was determined by the method of correlation analysis (Pearson's correlation coefficients), and the obtained coefficients tested by t-test for significance level of 0.05% and 0.01%. The obtained results are presented in Tables 3-11 and Figures 1-6.

## **RESULTS AND DISCUSSION**

### **Plant height**

The results showed that there was a significant difference between rye genotypes for plant height trait at 0.05% level in two years, Tab. 3, Fig. 1. There was a significant difference for the mean of plant height between two years. Genotype G1 had a statistically significantly higher studied parameter in 2019/2020 compared to genotype G2 in 2018/2019 (130 cm) and 2019/2020 (134.00 cm) and compared to genotype G1 in 2018/2019 (129.33 cm) (Table 3, Figures 1a, 1b). There were no statistically significant differences between the studied genotypes for the studied parameter,  $p > 0.05$ . The genotype G1 had plants height of 135.17 cm on average in both trial years, while the height of plants for genotype G2 was 132 cm, Table 3.

Based on the analysis of variance, it can be concluded that there are highly significant differences in rye plants height between tested year ( $F_{\text{exp}} = 6.330^*$ ) and no significant differences at studied genotypes (Table 4).



**Table 3.** Productivity parameters of rye genotypes, Serbia, 2018/2019-2019/2020.

Parameter	Genotype	Plant height, cm	Spike length, cm	Green biomass yield, t ha <sup>-1</sup>
2018/2019	G1	129.33±10.06	13.66±0.57	25.08±0.67
2019/2020		141.00±1.00	14.10±0.17	25.73±0.15
Average		135.16±9.04	13.88±0.44	25.41±0.56
2018/2019	G2	130.00±1.00	11.66±1.03	23.13±0.25
2019/2020		134.00±3.60	11.67±1.52	21.77±0.25
Average		132.00±3.22	11.67±0.57	22.45±0.78
Average 2018/19		129.66±6.40	12.66±1.50	24.11±1.16
Average 2019/20		137.50±4.50	12.88±1.38	23.75±2.18
Average 2018/19-2019/20		133.58±6.68	12.78±1.38	23.93±1.67

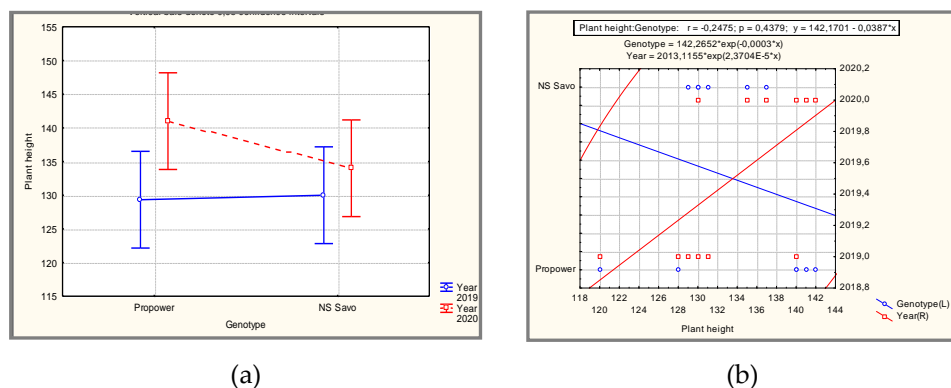
Parameter	Genotype		Year		G x Y	
	0.5	0.1	0.5	0.1	0.5	0.1
Plant height	7.180 <sup>ns</sup>	10.446 <sup>ns</sup>	7.180*	10.446*	10.154*	14.773*
Spike length	1.159*	1.686*	1.159 <sup>ns</sup>	1.686 <sup>ns</sup>	1.639*	2.384*
Green biomass yield	0.519*	0.756*	0.519 <sup>ns</sup>	0.756 <sup>ns</sup>	0.735*	0.340*

**Table 4.** ANOVA for rye plant height.

Effect	SS	Degr. of Fr.	MS	F	p
Intercept	214134.0	1	214134.0	7362.770**	0.000
Genotype	30.1	1	30.1	1.034 <sup>ns</sup>	0.339
Year	184.1	1	184.1	6.330**	0.036
G x Y	44.1	1	44.1	1.516 <sup>ns</sup>	0.253
Error	232.7	8	29.1		

\*\* significant at 0.01; <sup>ns</sup> - not significant.

The interaction of the studied factors (G × Y) showed no significant affect in rye plants height (p>0.05). Rye (*Secale cereale* L.) is an ideal crop for agricultural grain production in regions with less fertile and sandy soils, while post-harvest residues (biomass) can be successfully used for biogas production. The amount of precipitation in the vegetation season 2019 was less compared to the 2020 year of the study as well as in relation to the multiannual average. The amount of precipitation was significantly below the average, which represented poor conditions for the growth and development of plants in all pheno-phases (Table 3). Variations in the temperature and the amount of precipitation during vegetation period rye are the most important factors of the yield instability.



**Figure 1.** (a) Interaction year × genotype for plant height of rye genotypes; (b) Interaction genotype × year for plant height of rye genotypes, 2018/19-2019/20.

In the Serbia, high temperatures and the water deficiency during June resulted in grain yield decrease in many crops (Cakić and Stamenković, 2009; Hübner *et al.*, 2011). The yields are strongly modified by the environment of different temperatures and weather conditions. The drought has become a main limiting factor of the world plant production (Hübner *et al.*, 2011; Glamočlija *et al.*, 2015; Rajičić *et al.*, 2020; Dražić *et al.*, 2021). The present results confirm the opinion of many authors that the traits analyzed are genetically determined but are strongly modified by the environment and weather conditions (Popović *et al.*, 2020a).

The plant height was significantly influenced by the year and the  $G \times Y$  interaction (Table 1). Plant height has positive correlation with yield indicating that taller rye plants have higher yield (Table 8). Plant height were positively correlated with grain yield in the dry environments.

Plant height is one of the critical traits affected by drought in cereals. Low moisture reduces photosynthesis and metabolite/nutrient translocation in wheat, especially during the stem elongation stage, resulting in reduced height (Nsair *et al.*, 2020). The results of the field experiments indicated that there was variation for grain yield under drought stress among genotypes. The introduction of breeding programs for stress conditions is likely to increase in view of the predicted increase in the occurrence of high temperatures and droughts (Spyridonidis *et al.*, 2020; Milanović *et al.*, 2020).

The circular economy is an approach that integrates the economy, the waste management system and protects the environment. The goal of the circular economy is to optimize the existing system and increase welfare. According to the results of the research, which are stated by British authors, if the straw of cereals, grown only in the area of the eastern part of the Midlands, were used to obtain biofuels, the amount of obtained energy would cover about 1.5% of British consumption. Cereals as energy sources in the function of circular economy solution for the use of cereal straw should be the subject of further research. Straw can also be used to obtain liquid biofuels (ethanol) because it has large

amounts of carbohydrates. Today, in addition to the requirements for fuel quality, there are increasing requirements for low exhaust emissions of toxic gases and obtaining fuel from renewable energy sources (Dražić *et al.*, 2021).

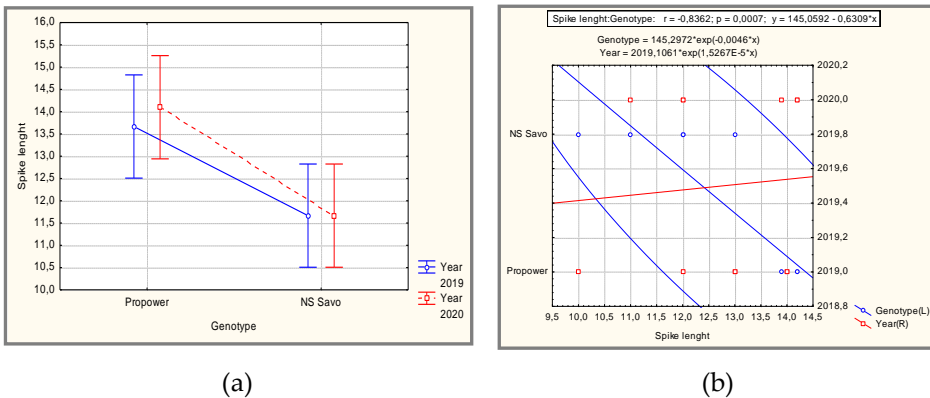
**Spikes length of rye genotypes**

Genotype had a statistically significant effect on spike length in plants of studied rye genotypes,  $p < 0.05$ . There were no statistically significant differences between the studied years and interactions of year  $\times$  genotype for the tested parameter,  $p > 0.05$ , Tables 3, 5. The mean of spike lengths in genotypes G1 and G2 was about 13.88 and 11.66 cm, respectively. The more favourable year for the tested parameters was 2019/2020 (12.88 cm) compared to 2018/2019 (12.67 cm), Tables 3, 5.

**Table 5.** ANOVA for spike length, 2018/2019-2019/2020.

Effect	SS	Degr. of Fr.	MS	F	p
Intercept	1958.4	1	1958.40	2585.35**	0.000
Genotype	14.74	1	14.74	19.46**	0.000
Year	0.14	1	0.14	0.19 <sup>ns</sup>	0.670
G x Y	0.14	1	0.14	0.19 <sup>ns</sup>	0.670
Error	6.06	8	0.76		

\*\* indicate significance different at 0.01; <sup>ns</sup> - not significant.



**Figure 2.** (a) Interaction year  $\times$  genotype for spike length; (b) Interaction genotype  $\times$  year for spike length, 2018/2019-2019/2020.

In 2019/2020, genotype G1 (14.10 cm) had a statistically significantly higher tested parameter in relation to the G2 genotype in 2018/2019 (11.66 cm) and 2019/2020 (11.67 cm), and in comparison to the G1 in 2018/2019 (13.66 cm) (Tables 3, Fig. 2a, 2b).

Based on the analysis of variance, it can be concluded that there are highly significant differences in the rye length of spikes between tested genotypes

( $F_{\text{exp}}=19.46^{**}$ ) and no significant differences at studied years (Table 3). There was no significant year  $\times$  genotype interaction for length of spikes in studied genotypes ( $p>0.05$ ). The present results confirm the opinion of many authors that the traits analyzed are genetically determined but are strongly modified by environment, of different temperatures and weather conditions (Popović *et al.*, 2020a). Interactions year  $\times$  genotype had a statistically significant effect on spike length studied rye genotypes (Table 3).

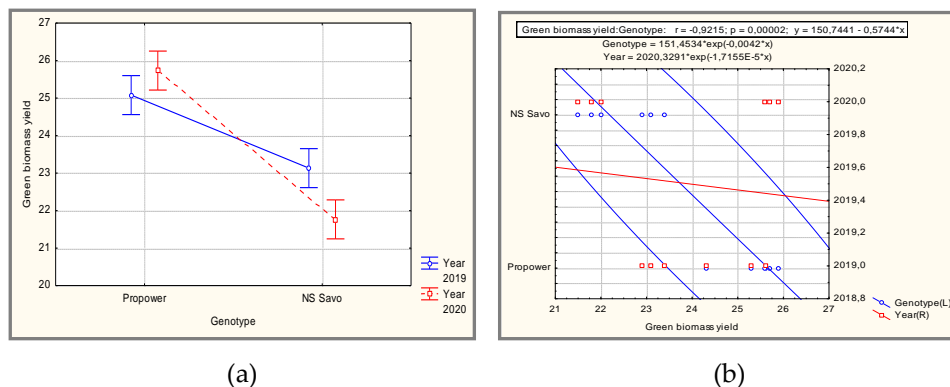
### Yield of green biomass

Genotype and genotype  $\times$  year interaction had a statistically significant effect on green biomass yield,  $p<0.05$  and  $p<0.01$  and no statistically significant differences between the studied years,  $p >0.05$ .

**Table 6.** ANOVA for green biomass yield

Effect	SS	Degr. of Fr.	MS	F	p
Intercept	6870.78	1	6870.78	45143.10	0.0000
Genotype	26.23	1	26.23	172.31**	0.0000
Year	0.38	1	0.38	2.51 <sup>ns</sup>	0.152
G x Y	3.060	1	3.060	20.11*	0.002
Error	1.218	8	0.152		

\* and \*\* indicate significance different at 0.05 and 0.01; ns: not significant.



**Figure 3.** (a) Interaction year  $\times$  genotype for green biomass yield; (b) Interaction genotype  $\times$  year for green biomass yield, 2018/2019-2019/2020.

The G1 genotype had, on average, for both studied years a statistically significantly higher yield of green biomass ( $25.73 \text{ t ha}^{-1}$ ) compared to the G2 genotype ( $23.75 \text{ t ha}^{-1}$ ). The most favourable year for the studied parameter was 2018/2019 ( $24.11 \text{ t ha}^{-1}$ ) compared to 2019/2020 ( $23.75 \text{ t ha}^{-1}$ ), Tables 3, 6.

Genotype G1 had a statistically significantly higher studied parameter in 2019/2020 ( $25.73 \text{ t ha}^{-1}$ ) compared to genotype G2 in 2019/2020 ( $21.77 \text{ t ha}^{-1}$ ) and 2018/2019 ( $23.13 \text{ t ha}^{-1}$ ) and in relation to the genotype G1 in 2018/2019 ( $25.08 \text{ t ha}^{-1}$ )

ha<sup>-1</sup>) (Tables 3, 6; Fig. 3). Based on the analysis of variance, it can be concluded that there are highly significant differences in the rye green biomass yield between tested genotypes ( $F_{\text{exp}}=172.31^{**}$ ). The interaction of the studied factors (G×Y) exhibits was significant affect in plants biomass yield ( $F_{\text{exp}}=20.11^*$ ), Tab. 6. Biomass is a renewable energy source derived from all plants and materials. Genotype and year × genotype interaction had a statistically significant effect on green biomass yield of tested rye genotypes, Table 3.

Based on the analysis of variance, it can be concluded that there are highly significant differences in biomass yield in regard to the genotype ( $F_{\text{exp}}=937.75^{**}$ ) and years ( $F_{\text{exp}}=28.07^{**}$ ) of investigation (Dražić *et al.*, 2021). Genotype, year and interaction of tested factors (G×Y) had a statistically significant effect on biogas yield. A selection for maximum dry biomass yield in rye breeding should indirectly improve also biogas and methane yield.

### Biogas yield

Genotype and genotype × year interaction had a statistically significant effect on biogas yield of tested rye genotypes,  $p<0.05$ . The G1 genotype had for both studied years a statistically significantly higher biogas yield ( $260.57 \text{ m}^3 \text{ t}^{-1}$ ) on average compared to the G2 genotype ( $214.58 \text{ m}^3 \text{ t}^{-1}$ ). The more favourable year for the studied parameter was 2018/2019 ( $237.85 \text{ m}^3 \text{ t}^{-1}$ ) compared to 2019/2020 ( $237.30 \text{ m}^3 \text{ t}^{-1}$ ), but the difference was not significant (Tables 7, 8).

**Table 7.** Productivity parameters of rye, Serbia, 2018/2019-2019/2020.

Parameter	Genotype	Biogas yield $\text{m}^3 \text{ t}^{-1} \text{ fm}$	Methane yield $\text{Nm}^3 \text{ ha}^{-1} \text{ dm}$	Methane content, %
2018/2019	G1	260.03±0.15	245.03±2.82	56.13±0.11
2019/2020		261.10±0.75	258.13±10.03	56.43±0.55
Average		260.56±0.76	251.58±9.74	56.28±0.39
2018/2019	G2	215.66±0.41	231.00±4.70	52.93±1.19
2019/2020		213.50±1.30	228.03±1.71	52.60±0.70
Average		214.58±1.46	229.52±3.56	52.77±0.89
Average 2018/19		237.85±24.30	238.02±8.43	54.53±1.90
Average 2019/20		237.30±26.08	243.08±17.69	54.52±2.17
Average 2018/19- 2019/20		237.58±24.03	240.55±13.48	54.53±1.95

\*fm - fresh biomass; dm - dry biomass

Parameter	Genotype		Year		G x Y	
	0.5	0.1	0.5	0.1	0.5	0.1
Biogas yield	1.043*	1.518*	1.043 <sup>ns</sup>	1.518 <sup>ns</sup>	1.475*	2.147 <sup>ns</sup>
Methane yield	7.701*	11.205*	7.701 <sup>ns</sup>	11.205 <sup>ns</sup>	10.891*	15.845*
Methane content	0.994*	1.446*	0.994 <sup>ns</sup>	1.446 <sup>ns</sup>	2.045*	2.045 <sup>ns</sup>

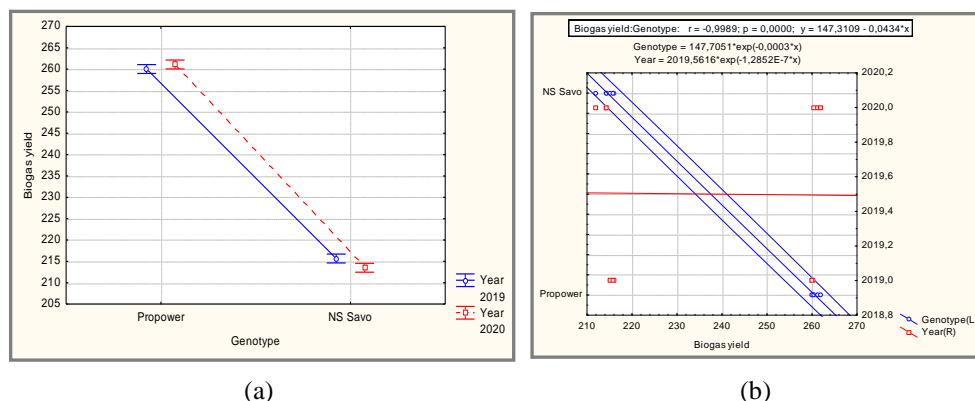
Based on the analysis of variance, it can be concluded that there are highly significant differences in the rye biogas yield between tested genotypes ( $F_{\text{exp}} =$

10328.10\*\*) and no significant differences in tested parameter at studied years. The interaction of the studied factors ( $G \times Y$ ) exhibits was significant high affect in biogas yield ( $F_{\text{exp}}=13.40^*$ ). Genotype G1 had a statistically significantly higher analyzed parameter in 2019/2020 ( $261.10 \text{ m}^3\text{t}^{-1}$ ) compared to genotype G2 in 2019/2020 ( $213.50 \text{ m}^3\text{t}^{-1}$ ) and 2018/2019 ( $216.66 \text{ m}^3\text{t}^{-1}$ ) (Fig. 4a, 4b).

**Table 8.** ANOVA for biogas yield.

Effect	SS	Degr. of Fr.	MS	F	p
Intercept	677302.10	1	677302.10	110279.10**	0.0000
Genotype	6343.40	1	6343.40	10328.10**	0.0000
Year	0.90	1	0.90	1.01 <sup>ns</sup>	0.2588
G x Y	7.80	1	7.80	13.40*	0.0072
Error	4.90	8	0.60		

\* and \*\* indicate significance different at 0.05 and 0.01; ns: not significant.



**Figure 4.** (a) Interaction year  $\times$  genotype for biogas yield; (b) Interaction genotype  $\times$  year for biogas yield, Serbia, 2018/2019-2019/2020.

Genotype and year  $\times$  genotype interaction had a statistically significant effect on biogas yield of studied rye genotypes, Table 7. Genotype G1 had a statistically significantly higher analyzed parameter in 2020 compared to genotype G2, Fig. 4a. Based on the analysis of variance, it can be concluded that there are highly significant differences in biogas yield in regard to the genotype ( $F_{\text{exp}}=3902.25^{**}$ ) and investigated years ( $F_{\text{exp}}=5.32^*$ ). The interaction of the investigated factors ( $G \times Y$ ) exhibits was no significant affect in yield (Dražić et al., 2021).

### Methane yield

Based on the analysis of variance, it can be concluded that there are highly significant differences in the rye methane yield between tested genotypes ( $F_{\text{exp}}=43.66^{**}$ ) and no significant differences at studied years. The interaction of

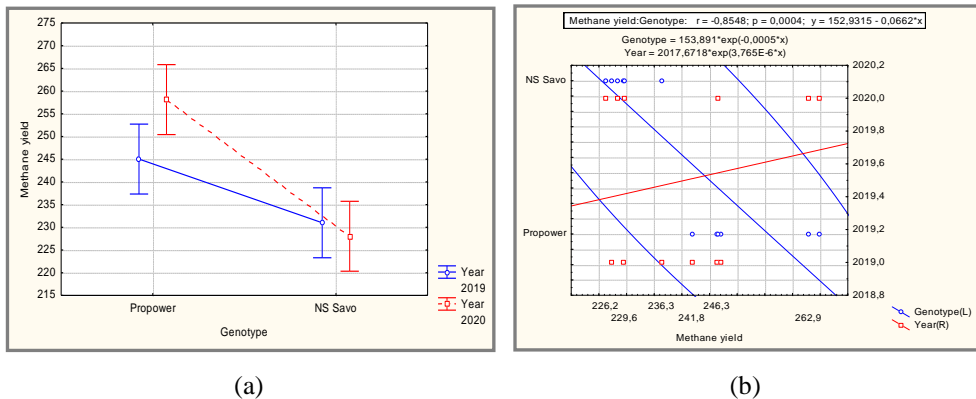
the studied factors (G×Y) exhibits was significant affect in methane yield ( $F_{exp}=5.79^*$ ), Table 9.

**Table 9.** ANOVA for methane yield.

Effect	SS	Degr. of Fr.	MS	F	p
Intercept	694371.50	1	694371.50	20752.20	0.0000
Genotype	1460.8	1	1460.8	43.66**	0.0001
Year	77.00	1	77.00	2.30 <sup>ns</sup>	0.16771
G x Y	193.60	1	193.60	5.79*	0.0428
Error	267.70	8	33.50		

\* and \*\* indicate significance different at 0.05 and 0.01; ns: not significant.

The G1 genotype had on average for both studied years a statistically significantly higher methane yield ( $251.58 \text{ m}^3 \text{ ha}^{-1}$ ) compared to the G2 genotype ( $229.52 \text{ m}^3 \text{ ha}^{-1}$ ).



**Figure 5.** (a) Interaction year × genotype for methane yield; (b) Interaction genotype × year for methane yield, 2018/2019-2019/2020.

The more favourable year for the analyzed parameter was 2019/2020 ( $243.08 \text{ m}^3 \text{ ha}^{-1}$ ) compared to 2018/2019 ( $238.01 \text{ m}^3 \text{ ha}^{-1}$ ), the difference between the years was not significant. In 2019/2020, the genotype G1 had a statistically significantly higher ( $258.13 \text{ m}^3 \text{ ha}^{-1}$ ) tested parameter compared to the genotype G2 in 2019/2020 ( $228.03 \text{ m}^3 \text{ ha}^{-1}$ ) and 2018/2019 ( $231.00 \text{ m}^3 \text{ ha}^{-1}$ ) and genotype G1 in 2018/2019 ( $245.03 \text{ m}^3 \text{ ha}^{-1}$ ) (Tables 7, 9; Figures 5a, 5b).

Genotypes and G × Y interaction had a substantial influence on the expression of rye methane yield, Tables 7 and 9. Maximum methane yield per hectare is the main aim of the farmer. Significant ( $p < 0.05$ ) genotypic variation was found for dry matter yield, specific gas yield and methane yield among the 25 genotypes. Ranges were achieved for dry matter yield (0% water content) and methane yield amounting to  $2.9 \text{ t ha}^{-1}$  and  $840 \text{ m}^3 \text{ ha}^{-1}$  respectively, combined

with moderate to high heritabilities (0.71–0.98) (Nsair *et al.*, 2020). Anaerobic digestion is an established technology, used to treat a wide variety of organic wastes. It is one of several biological processes that deliver economic and environmental benefits (i.e., producing bioenergy and/or biochemical while treating the organic fraction of waste) (Nsair *et al.*, 2020; Dražić *et al.*, 2021).

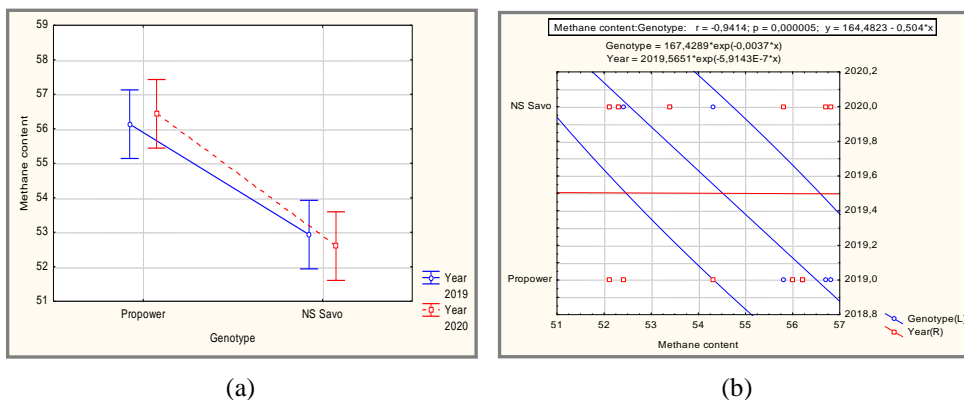
### Methane content

Based on the analysis of variance, it can be concluded that there are highly significant differences in the methane content between tested genotypes ( $F_{\text{exp}}=66.55^{**}$ ) and no significant differences during studied years and the interaction of the studied factors ( $G \times Y$ ), Table 10. Genotype had a statistically significant effect on the methane content of the studied rye genotypes,  $p<0.05$ . The G1 genotype had on average significantly higher methane content (56.28 %) for both studied years compared to the G2 genotype (52.77 %).

**Table 10.** ANOVA for methane content.

Parameter	SS	Degr. of Fr.	MS	F	p
Intercept	35675.71	1	35675.71	63992.30	0.0000
Genotype	37.10	1	37.10	66.55**	0.0000
Year	0.00	1	0.00	0.01 <sup>ns</sup>	0.16771
G x Y	0.30	1	0.30	0.54 <sup>ns</sup>	0.0428
Error	4.46	8	0.56		

\* and \*\* indicate significance different at 0.05 and 0.01; ns: not significant.



**Figure 6.** (a) Interaction  $Y \times G$  for methane content (b) Interaction  $G \times Y$  for methane content, 2018/2019-2019/2020.

The more favourable year for the tested parameter was 2018/2019 (54.53%) compared to 2019/2020, where the difference between the years was not significant. In 2019/2020, the genotype G1 had (54.53%) statistically



significantly higher tested parameter compared to the genotype G2 in 2019/2020 (52.60%) and 2018/2019 (52.93%), Tables 7, 10.

Application of varietal production technology improved increasing the yields (Popović *et al.*, 2020b). The biogas production technology has improved over the last years for the aim of reducing the costs of the process, increasing the biogas yields, and minimizing the greenhouse gas emissions (Spyridonidis *et al.*, 2020).

### Correlations of tested traits.

Correlations of the tested traits of rye varieties are shown in Table 11. A positive statistically very significant correlation was achieved between green biomass yield and spike length ( $r=0.82^{**}$ ), green biomass yield and biogas yield ( $r=0.93^{**}$ ), green biomass yield and methane content ( $r=0.90^{**}$ ).

**Table 11.** Correlation of tested traits of rye genotypes, 2018/2019-2019/2020.

Variable	Plant height	Spike length	Green biomass yield	Biogas yield	Methane yield	Methane content
PH	1.00	0.30 <sup>ns</sup>	0.26 <sup>ns</sup>	0.25 <sup>ns</sup>	0.43*	0.22 <sup>ns</sup>
SL	0.30 <sup>ns</sup>	1.00	0.82 <sup>**</sup>	0.83 <sup>**</sup>	0.84 <sup>**</sup>	0.88 <sup>**</sup>
GBY	0.26 <sup>ns</sup>	0.82 <sup>**</sup>	1.00	0.93 <sup>**</sup>	0.87 <sup>**</sup>	0.90 <sup>**</sup>
BY	0.25 <sup>ns</sup>	0.83 <sup>**</sup>	0.93 <sup>**</sup>	1.00	0.86 <sup>**</sup>	0.94 <sup>**</sup>
MY	0.43*	0.84 <sup>**</sup>	0.87 <sup>**</sup>	0.86 <sup>**</sup>	1.00	0.85 <sup>**</sup>
MC	0.22 <sup>ns</sup>	0.88 <sup>**</sup>	0.90 <sup>**</sup>	0.94 <sup>**</sup>	0.85 <sup>**</sup>	1.00

<sup>ns</sup> - not significant; \* and \*\* indicate significance different at 0.05 and 0.01; PH-Plant height; SL-Spike length; GBY-Green biomass yield; BY-Biogas yield; MY-Methane yield; MC-Methane content.

A positive statistically significant correlations were achieved between the methane yield and plant height ( $r=0.43^*$ ), Table 11.

A positive and statistically high significant correlations were achieved between spike length and biogas yield ( $r=0.83^{**}$ ), and spike length and methane yield ( $r=0.84^{**}$ ), as well as the spike length and methane content ( $r=0.88^{**}$ ). A positive and statistically high significant correlations were achieved between the methane content and biogas yield ( $r=0.94^{**}$ ), the methane content and methane yield ( $r=0.85^{**}$ ), Table 11.

The study results indicate that GBY - green biomass yield in all vegetation seasons was positively and highly significantly correlated with SL-spike length, BY-biogas yield, MY- methane yield and MC-methane content, Table 11.

The biomass yield (BY) was in positive very significant dependence on the spike length ( $r=0.83^{**}$ ) and BY was in positive very significant dependence with the methane yield ( $r=0.86^{**}$ ). Commercial production should be economically and environmentally friendly so that renewable fuels could be an adequate replacement for fossil fuels (Milanović *et al.*, 2020; Popović *et al.*, 2020a). The correlative dependence of the GY- grain yield in the vegetation seasons was

positive and highly significant with BY-biogas yield as established by Popović *et al.*, (2020a) and Rakašćan *et al.*, (2021).

The environment can have a crucial influence on plants production in particular regions, even more so when production environments are different from optimum breeding environments (Janković *et al.*, 2016; Popović *et al.*, 2020b; Tmušić *et al.*, 2021). Positive highly significant correlation was obtained between grain yield and biogas yield ( $r=0.98$ ). Then, positive significant correlation were between grain yield and plant height ( $r=0.76$ ) and grain yield and precipitation (Popović *et al.*, 2020a).

Plant height were positively correlated with grain yield in the dry environments (Mackay *et al.*, 2009; Sarto *et al.*, 2017; Božović *et al.*, 2020; Mihailovic *et al.*, 2020; Siekmann *et al.*, 2021). The results of the field experiments indicated that there was variation for grain yield under drought stress among genotypes. The introduction of breeding programs for stress conditions is likely to increase in view of the predicted increase in the occurrence of high temperatures and droughts (Sahebi *et al.*, 2001; Manoj *et al.*, 2014; Lakew *et al.*, 2021).

The means value of length of rye spikes were significantly correlated for all analyzed traits (Table 11). The majority of the studied traits were correlated to each other. The strongest correlations were observed between GBY and BGY, GBY and MC, BGY and MC, GBY and MY, as well as between MY and MC. Markedly weaker correlations were noticed between PH and the majority of the other traits, Table 11. Grain quality, as well as agronomic important traits controlling plant height, heading date, thousand-grain weight, or yield, reveal a continuous phenotypic variation and are genetically controlled by a network of multiple and interacting loci (Popović *et al.*, 2021; Janković *et al.*, 2016). Analysis of variance (ANOVA) revealed that genotypic effects were statistically significant ( $p < 0.05$ ) for all traits.

Achieving high rye grain yield and quality demands a proper choice of the genotype and by applying the optimal production technology. Genotypes of the new generation exhibit a high degree of tolerance against temperature stress. Two genotypes were selected as the object of research in this study: G1 - Propower and G2 - NS Savo. High grain yield of genotype NS Savo in years with different environmental conditions of over  $8.84 \text{ t ha}^{-1}$ , excellent tolerance to low temperatures, resistance to the most important diseases and lying down, allows this cultivation varieties and in less favorable conditions and achieving very high yields (Đurić *et al.*, 2021).

Rye together with wheat is the most important bread grain. Rye bread stays fresh for a long time, it is rich in vitamins A, B and E, and since it is great digestibility is recommended for the diet of diabetics. Rye grain is the raw material for production starch and the production of spirits. Rye bread and bakery products have an increasing role in a healthy diet as well convalescent diets and people with elevated blood pressure. Rye is a good bread and bioenergy crop.

## CONCLUSIONS

Thanks to the development of new technologies for processing bio-waste into energy sources, the growth rate of the use of alternative fuels is growing significantly. Rye is an excellent raw material for the production of healthy-safe food, but also for the production of biofuels. Our study shows that there are genotypic differences among rye for the biogas production and rye biomass yield. Genotype and genotype x year interaction had a statistically significant effect on yield of biogas and methane and methane content in the studied rye genotypes. Genotype G1 had on average for both studied years a statistically significantly higher biogas yield compared to genotype G2. Genotype G1 was selected as a bioenergy crop and is more suitable for biofuel production. A positive statistically very significant correlation was achieved between green biomass yield and spike length ( $r=0.82^{**}$ ), green biomass yield and biogas yield ( $r=0.93^{**}$ ), green biomass yield and methane content ( $r=0.90^{**}$ ).

The review concludes that there is a need for comprehensive high throughput phenotyping of physio-morphological traits that is growth stage-based to improve the efficiency of breeding highly quality drought-tolerant rye but also for the production of highly productive genotypes for biofuel. Although there are achievements, challenges in rye production remain. A selection rye genotypes with maximum dry biomass yield in rye breeding should indirectly improve also biogas and methane yield. Breeding of varieties with high yield potential biomass is desirable for biogas production, and high grain yield potential with high grain qualities is necessary to further advance rye to high-performance crop with different types of end-use.

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