

# Effect of L-proline and L-glutamic acid on productivity of winter rapeseed

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## ABSTRACT

A higher level of fertilizers can have negative effects on the environment; therefore, selection of environmentally friendly preparations to increase plant productivity is very important. The aim of this study was to evaluate the effect of exogenously applied amino acids L-proline (30 mM L<sup>-1</sup>) and L-glutamic acid (1.5 M L<sup>-1</sup>) on the productivity of winter rapeseed (*Brassica napus* L. subsp. *oleifera* (Delile) Sinskaya) measuring number of siliques per plant, number of seeds per silique, 1000-seed weight, seed yield, crude fat content and crude fiber content. Treatments were applied at one or two growth stages of rapeseed. Higher 1000-seed weight and seed yield were obtained with L-glutamic acid in autumn and spring, while the higher number of siliques per plant was observed without amino acid application. Application of both amino acids led to an increase in seed yield of winter rapeseed from 3.5% to 11.8%. Principal component analysis showed that the first factor was highly and positively correlated with seed yield, 1000-seed weight, and crude fat and negatively with crude fiber, while the second factor was highly and positively correlated with silique per plant and negatively with seeds per silique. Higher values of crude fat content, seed yield, and 1000-seed weight were associated with application of glutamic acid on winter rapeseed, while crude fiber content was related to the application of proline. The study showed that higher 1000-seed, seed yield weight and crude fat content in seeds of winter rapeseed ( $P < 0.05$ ) were obtained spraying plants with 1.5 M L<sup>-1</sup> L-glutamic acid solution in autumn and spring.

**Key words:** Amino acids, *Brassica napus*, crude fat, crude fiber, seed yield.

## INTRODUCTION

Rapeseed (*Brassica napus* L.) is an important oilseed species widespread in many parts of the world. Rapeseed, as a raw material for vegetable oil production, is the most widely produced oilseed in the European Union, ranking second in the world. According to the European Commission, in 2019-2020, rapeseed areas in the European Union amounted to 5.18 million ha, 15.4 million tons of rapeseed (Committee for the Common Organization of Agricultural Markets, 2020). Durrett et al. (2008) state that rapeseed cultivation yields almost twice as much vegetable oil per hectare as soybean cultivation. The demand for rapeseed oil is increasing due to the increasing use of renewable energy sources in recent decades (Zhang et al., 2010; Klugmann-Radziemska and Ciunel, 2013; Ozturk, 2014). In establishing the 2030 climate and energy policy strategy, the European Council emphasized the importance of reducing greenhouse gas emissions and the risks associated with the transport sector's dependence on fossil fuels (European Parliament and the Council of the European Union, 2015). To reduce environmental pollution, rapeseed oil is increasingly used in the production of fuels and lubricants around the world. Biodiesel has been found to significantly reduce greenhouse gas emissions compared to conventional diesel (Ettl et al., 2020). In addition, rapeseed oil is used as a good quality lubricating oil (Sulek et al., 2010). Oil from varieties with a high content of erucic acid is used for the production of behenic acid and behenic alcohol (Zanetti et al., 2009).

A number of fertilizers and pesticides are often used in the cultivation of agricultural crops (Bulgari et al., 2015), but high levels of N fertilizers can have negative effects on the environment. Leakage of nitrates into waterbodies can increase the amount of nitric oxide, which increases the greenhouse effect (Mattner et al., 2013). While some researchers argue that environmental damage can be reduced by using exogenous plant growth regulators (Kazan, 2015; Khan et al., 2015), research has shown that some growth regulators in waterbodies pose a threat to aquatic ecosystems (Wang et al., 2011a). In addition, improper use of growth regulators on agricultural plants affects mammalian reproduction and fertility as it may increase estrogenic activity (Sorensen and Danielsen, 2006). Therefore, it is vital that environmentally friendly preparations that are non-toxic to humans are selected for applications to increase plant productivity.

Biological preparations (biostimulants), which are increasingly being used in agricultural plant agrotechnologies, activate physiological processes, increase the efficiency of nutrient use, and promote plant growth and development, thereby allowing for a reduction in the use of fertilizers (Kunicki et al., 2010; Ziosi et al., 2013). Biostimulants are extracts obtained from organic raw materials containing bioactive compounds. The most common components of biostimulants are mineral elements, humic substances (HS), vitamins, amino acids, chitin, chitosan, protein hydrolysates, and poly- and oligosaccharides (Lotfi et al., 2015; Du Jardin, 2015; Moreno-Hernández et al., 2020). Studies have shown that biostimulants increase the amount of photosynthetic pigments in plants, intensify the efficiency of photosynthesis and carbohydrate biosynthesis (Ertani and Nardi, 2013), activate physiological processes, and increase the efficiency of nutrient use, promoting plant development (Bulgari et al., 2015; Bardgett and Gibson, 2017).

To the best of our knowledge, studies about the effect of L-proline and L-glutamic acids on the productivity of winter rapeseed have not been previously reported. Therefore, the aim of this study was to investigate the effect of exogenously applied L-proline and L-glutamic acids on the number of siliques per plant, number of seeds per silique, 1000-seed weight, seed yield, crude fat content, and crude fiber content of winter rapeseed.

## MATERIALS AND METHODS

The research was carried out at the Experimental Station of Vytautas Magnus University Agriculture Academy, Kaunas (54°53' N, 23°50' E), Lithuania, from 2014 to 2017.

The winter rapeseed (*Brassica napus* L. subsp. *oleifera* (Delile) Sinskaya) 'Cult' was evaluated in an Endocalcaric Luvisol (IUSS Working Group WRB, 2015). Winter wheat was the previous crop. Soil agrochemical properties (data averages of 2014-2016) were: soil pH 6.97, 2.51% humus; plant-available nutrients in soil: 242 mg P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup>, 124 mg K<sub>2</sub>O kg<sup>-1</sup>, 1.52% total N.

Soil was fertilized with N<sub>185</sub>P<sub>40</sub>K<sub>60</sub> (N<sub>14</sub> in autumn before sowing, N<sub>171</sub> in spring). Winter rapeseed was sown on 20 August 2014, 25 August 2015, and 20 August 2016 using a Multidrill M 300 (Rabe, Bad Essen, Germany). Seed rate was 5 kg ha<sup>-1</sup>. After sowing, the rapeseed was sprayed with herbicide metazachlor at 2.0 L ha<sup>-1</sup>, twice with insecticide thiacloprid and deltamethrin at 0.8 and 0.6 L ha<sup>-1</sup>, and with fungicide tebuconazole 2 times at 0.5 L ha<sup>-1</sup> in autumn and 1.0 L ha<sup>-1</sup> in summer. The initial plot size was 84 m<sup>2</sup>, and that of the recorded plot was 20 m<sup>2</sup>. Experimental plots were arranged according to a split-plot design. The research was performed in four replicates. The treatments were applied at one or two growth stages of rapeseed. The first application was at the stage of 4-5 leaves unfolded (BBCH 14-15), and the second application was at the stage of 6-8 leaves unfolded (BBCH 16-18) or at the stage of yellow floral bud (BBCH 59).

The treatments applied were: Control without amino acids; 30 mM L<sup>-1</sup> L-proline sprayed in autumn at the stage of 4-5 leaves unfolded (BBCH 14-15) (30 ProA); 30 mM L<sup>-1</sup> L-proline sprayed in autumn at BBCH 14-15 and at the stage of 6-8 leaves unfolded (BBCH 16-18) (30 ProA + 30 ProA); 1.5 M L<sup>-1</sup> L-glutamic acid sprayed in autumn at BBCH 14-15 (1.5 GluA); 1.5 M L<sup>-1</sup> L-glutamic acid sprayed in autumn at BBCH 14-15 and BBCH 16-18 (1.5 GluA + 1.5 GluA); 30 mM L<sup>-1</sup> L-proline sprayed in autumn at BBCH 14-15 and at the stage of yellow floral bud (BBCH 59) (30 ProA + 30 ProS); and 1.5 M L<sup>-1</sup> L-glutamic acid sprayed in autumn at BBCH 59 (1.5 GluA + 1.5 GluS).

Winter rapeseed was sprayed with the test amino acid solutions using a handheld sprayer (Star 12 Green, Osatu, Gipuzkoa, Spain) after first allowing dew to evaporate at air temperature below 20 °C.

Soil agrochemical properties were determined at the Agrochemical Research Laboratory of the Lithuanian Research Centre for Agriculture and Forestry before sowing of winter rapeseed. For experimental purposes, samples were taken from the 0-25 cm soil layer of each experimental plot using a soil sampling auger. Soil pH was determined potentiometrically in

1 N KCl extract, and available P (P<sub>2</sub>O<sub>5</sub>) and K (K<sub>2</sub>O) (mg kg<sup>-1</sup> soil) were estimated by the Egner-Riehm-Domingo (A-L) method, and organic C was determined using an analyzer Multi EA 4000 (Analytic Jena AG, Jena, Germany) instrument. The humus content was calculated by multiplying the organic C content by a factor of 1.724.

### Weather conditions

Weather conditions during the trials were obtained from the Kaunas agrometeorological station, Lithuania (Table 1). In May and June 2016, the average monthly temperatures were higher in comparison to the same periods in 2015 and 2017, as was the case with long-term average temperature. The average monthly precipitation in May was lower compared to long-term average, especially in 2017. June and August 2015 were very dry, and average monthly precipitation was 3.8 and 11.6 times lower, respectively, in comparison with the long-term average.

### Yield and quality variables

Plants were taken from a 1 m<sup>2</sup> area of each experimental plot for the evaluation of the structural variables of yield (number of pods, number of seeds per pod, and 1000-seed weight) and seed yield before harvesting. Qualitative variables of seeds (raw fat and crude fiber contents) were evaluated at the Laboratory of Raw Materials for Food Zootechnical and Agronomical Analyses, Vytautas Magnus University Agriculture Academy. Raw fat content (%) was determined using a Soxhlet extractor by extraction with petroleum ether, and crude fiber content (%) was determined by the Henneberg-Stohmann method.

### Statistical analysis

Statistical analysis of the experimental data involved ANOVA using software package Statistica, version 7 (TIBCO Software, Palo Alto, California, USA). The mean values of the number of siliques per plant, number of seeds per silique, 1000-seed weight, seed yield, amount of crude fat and crude fiber content, and standard error (SE) were calculated on the basis of the number of independent replicates. The effect of factors (amino acids and year) and their interaction with

**Table 1. Air temperature and precipitation during experiments.**

Months	Average monthly temperature, °C				
	2014	2015	2016	2017	Long-term average
January		-0.40	-7.09	-3.67	-4.80
February		0.20	0.58	-1.48	-4.20
March		4.60	2.07	3.72	-0.70
April		7.10	7.43	5.61	6.10
May		11.40	15.73	12.87	12.30
June		15.40	17.21	15.35	15.60
July		17.40	17.89	16.77	17.60
August	17.90	20.30	16.90		16.60
September	13.50	14.30	13.48		12.20
October	7.50	6.23	5.30		6.80
November	2.80	4.85	2.80		1.50
December	0.90	2.60	3.40		-3.30
Months	Average monthly precipitation, mm				
	2014	2015	2016	2017	Long-term average
January		74.40	41.60	18.40	30.80
February		12.80	68.40	31.30	27.60
March		45.70	47.20	53.10	32.50
April		46.00	41.20	73.70	38.40
May		43.80	36.40	10.50	53.80
June		16.40	83.90	80.20	62.60
July		72.40	162.90	79.60	81.20
August	112.30	6.90	114.90		80.30
September	20.70	56.60	22.50		52.60
October	90.30	18.20	101.50		19.60
November	29.30	95.60	101.50		46.10
December	49.30	61.30	77.90		36.10

investigated variables were studied by two-way ANOVA. Fisher's least significant difference (LSD) test was carried out with the significance level of  $p < 0.05$ . Linear correlation and regression analyses were performed to determine the strength and character of the relationships between variables at a probability level of 95%. Principal component analysis was performed using XLSTAT software (XLSTAT, 2018Addinsoft, Paris, France) to analyze the association between applied amino acids and elements of yield structural variables such as crude fat and fiber content of winter rapeseed.

## RESULTS AND DISCUSSION

The effect of amino acids and year on the investigated variables of 1000-seed weight, seed yield, and raw fat content were calculated using ANOVA and the results are presented in Table 2. The application of exogenous amino acids inhibited the formation of winter rapeseed siliques.

The lowest number of siliques per plant was formed under the influence of 30 mM L<sup>-1</sup> L-proline solution sprayed twice in autumn (30 ProA + 30 ProA) and 30 mM L<sup>-1</sup> L-proline solution sprayed in autumn and spring (30 ProA + 30 ProS). Treatment 30 ProA + 30 ProS and 30 mM L<sup>-1</sup> L-proline in autumn (30 ProA) resulted in the significantly highest numbers of seeds per silique, 20.33 and 20.22 units respectively. The significantly highest 1000-seed weight was determined for plants sprayed with studied amino acids in autumn and spring, 1.5 GluA + 1.5 GluS and 30 ProA + 30 ProS. Application of L-glutamic acids in autumn and spring (1.5 GluA + 1.5 GluS) resulted in the highest seed yield and crude fat content and lowest crude fiber content. The growing seasons had different effects on the evaluated variables. In 2015, significantly higher 1000-seed weight and seed yield were observed, while a significantly higher number of seeds per silique was determined in 2017. No influence of growing season on the number of siliques per plant, crude fat, or crude fiber content was observed. During 2015 to 2017, the highest number of siliques per plant was observed in the absence of amino acid application (Table 3).

The number of seeds per silique under the influence of amino acids varied in different years. In 2015, the highest number of seeds per silique was observed in 30 ProA, while in 2016 this occurred for 1.5 GluA + 1.5 GluS; the effects of exogenously applied amino acids on this variable were not determined in 2017. The significantly highest 1000-seed weight was found in 30 ProA + 30 ProS and 1.5 GluA + 1.5 GluS plants (2015 and 2017), while in 2016, application of the tested amino acids increased this variable independently of the concentration and application timing.

**Table 2. Effect of amino acids and year on number of siliques per plant, number of seeds per silique, 1000-seed weight, seed yield, crude fat content, and crude fiber content.**

Experimental factor	Siliques per plant	Seeds per silique	1000-seed weight	Seed yield	Crude fat content	Crude fiber content
	Units		g	t ha <sup>-1</sup>	%	
Amino acids						
Control	102.48a	19.58ab	5.17d	4.00c	46.23b	24.37a
30 ProA	92.23c	20.22a	5.33bcd	4.14bc	46.57b	23.80ab
30 ProA + 30ProA	89.91c	19.56ab	5.37bcd	4.21b	46.47b	23.27ab
1.5 GluA	95.67b	19.68ab	5.40bcd	4.37ab	47.40ab	23.13ab
1.5 GluA + 1.5 GluA	90.77c	19.12b	5.44bcd	4.43ab	46.87b	23.37ab
30 ProA + 30 ProS	89.33c	20.33a	5.59a	4.31ab	46.13b	23.97ab
1.5 GluA + 1.5 GluS	96.78b	20.02ab	5.60a	4.47a	48.47a	22.03b
Year						
2015	94.25a	20.08b	5.58a	4.58a	46.67ab	23.32a
2016	92.60a	18.65c	5.25c	3.89c	46.50ab	23.74a
2017	94.90a	20.63a	5.40b	4.36b	47.46b	23.17a

For each experimental factor, values in columns followed by different letters are significantly different at  $P < 0.05$  according to Fisher's test.

Control: Without amino acids; 30 ProA: sprayed with 30 mM L<sup>-1</sup> L-proline in autumn at the stage of 4-5 leaves unfolded (BBCH 14-15); 30 ProA + 30 ProA: sprayed with 30 mM L<sup>-1</sup> L-proline in autumn at BBCH 14-15 and at the stage of 6-8 leaves unfolded (BBCH 16-18); 1.5 GluA: sprayed with 1.5 M L<sup>-1</sup> L-glutamic acid in autumn at BBCH 14-15; 1.5 GluA + 1.5 GluA: sprayed with 1.5 M L<sup>-1</sup> L-glutamic acid in autumn at BBCH 14-15 and BBCH 16-18; 30 ProA + 30 ProS: sprayed with 30 mM L<sup>-1</sup> L-proline in autumn at BBCH 14-15 and at the stage of yellow floral bud (BBCH 59); 1.5 GluA + 1.5 GluS: sprayed with 1.5 M L<sup>-1</sup> L-glutamic acid in autumn at BBCH 59.

**Table 3. Effect of the exogenous amino acids on number of siliques per plant, number of seeds per silique, 1000-seed weight, seed yield, crude fat content, and crude fiber content.**

Treatments	Siliques per plant	Seeds per silique	1000-seed weight	Seed yield	Crude fat content	Crude fiber content
	Units		g	t ha <sup>-1</sup>	%	
2015						
Control	106.76a	20.2b	5.24bc	4.34c	46.3b	24.1a
30 ProA	91.19bc	21.4a	5.36bc	4.47bc	45.8b	23.9ab
30 ProA + 30 ProA	86.50c	20.1b	5.47b	4.44c	46.2b	22.5b
1.5 GluA	99.62b	19.8b	5.48b	4.65b	47.8a	22.9b
1.5 GluA + 1.5 GluA	89.98bc	18.6c	5.67b	4.65b	46.1b	24.2a
30 ProA + 30 ProS	86.13c	20.7ab	5.94a	4.64b	46.3b	23.7ab
1.5 GluA + 1.5 GluS	99.63b	19.7b	5.91a	4.86a	48.2a	22.1b
2016						
Control	98.35a	18.6ab	5.08b	3.60c	45.9b	24.2a
30 ProA	91.82b	18.7ab	5.29a	3.62c	46.3b	23.9ab
30 ProA + 30 ProA	89.64b	18.2b	5.28a	3.92b	45.9b	24.2a
1.5 GluA	91.85b	18.4b	5.33a	4.03ab	46.5ab	23.8ab
1.5 GluA + 1.5 GluA	90.92b	18.3b	5.28a	4.23a	46.9ab	23.1b
30 ProA + 30 ProS	90.31b	19.1ab	5.31a	3.80bc	45.9b	24.3a
1.5 GluA + 1.5 GluS	95.37ab	19.4a	5.26a	3.98b	48.1a	22.7b
2017						
Control	102.31a	19.9a	5.19b	4.03c	46.5b	24.8a
30 ProA	93.75b	20.6a	5.35ab	4.34b	47.6ab	23.6ab
30 ProA + 30 ProA	93.62b	20.4a	5.37ab	4.26bc	47.3b	23.1ab
1.5 GluA	95.58ab	20.8a	5.38ab	4.44ab	47.9ab	22.7ab
1.5 GluA + 1.5 GluA	91.43b	20.5a	5.40ab	4.41ab	47.6ab	22.8ab
30 ProA + 30 ProS	91.53b	21.3a	5.52a	4.50a	46.2b	23.9a
1.5 GluA + 1.5 GluS	95.36ab	20.9a	5.63a	4.56a	49.1a	21.3b

For each experimental factor, values in columns followed by different letters are significantly different at  $P < 0.05$  according to Fisher's test.

Control: Without amino acids; 30 ProA: sprayed with 30 mM L<sup>-1</sup> L-proline in autumn at the stage of 4-5 leaves unfolded (BBCH 14-15); 30 ProA + 30 ProA: sprayed with 30 mM L<sup>-1</sup> L-proline in autumn at BBCH 14-15 and at the stage of 6-8 leaves unfolded (BBCH 16-18); 1.5 GluA: sprayed with 1.5 M L<sup>-1</sup> L-glutamic acid in autumn at BBCH 14-15; 1.5 GluA + 1.5 GluA: sprayed with 1.5 M L<sup>-1</sup> L-glutamic acid in autumn at BBCH 14-15 and BBCH 16-18; 30 ProA + 30 ProS: sprayed with 30 mM L<sup>-1</sup> L-proline in autumn at BBCH 14-15 and at the stage of yellow floral bud (BBCH 59); 1.5 GluA + 1.5 GluS: sprayed with 1.5 M L<sup>-1</sup> L-glutamic acid in autumn at BBCH 59.

In 2015, the highest seed yield was obtained by 1.5 GluA + 1.5 GluS. The extra yield averaged 520 kg ha<sup>-1</sup>. In 2016, the yield of winter rapeseeds varied from 3.60 to 4.23 t ha<sup>-1</sup>. The highest seed yields were obtained by 1.5 GluA + 1.5 GluA, with an average extra yield of 380 kg ha<sup>-1</sup>. In 2017, the seed yield of winter rapeseed varied from 4.03 to 4.56 t ha<sup>-1</sup>. The highest seed yield was obtained by 1.5 GluA + 1.5 GluS plants. The extra yield was 530 kg ha<sup>-1</sup> on average.

The significantly highest crude fat content in seeds of winter rapeseed was determined by 1.5 GluA + 1.5 GluS plants. In many cases, exogenously applied amino acids resulted in a decrease in crude fiber content in winter rapeseed seeds.

The scientific literature indicates that seed yield of rapeseed is determined by the number of siliques (Yasari et al., 2008), number of seeds per silique (Wang et al., 2011b), and 1000-seed weight (Szczepanek et al., 2016). In our study, the results were analyzed using correlation and regression, and it was found that there was a direct, strong, and significant correlation between 1000-seed weight and seed yield ( $y = 0.93676x - 0.7959$ ;  $r = 0.83$ ).

Popko et al. (2018) investigated the influence of a product based on amino acids on winter rapeseed yield and grain quality. They found that application of the tested product increased grain yield by 5.4%-11.0%. Significantly increased yield of soybean under foliar application of biostimulant with amino acids was reported by Kocira (2019). The positive effect of exogenously applied amino acids on plant productivity also has been found in studies with tomato (Koukounararas et al., 2013), lettuce (Tsouvaltzis et al., 2014) and wheat (Sierras et al., 2016). Our research demonstrates that the application of the studied exogenous amino acids increased seed yield of winter rapeseed. Depending on the type of amino acid

and timing of application, seed yield of winter rapeseed increased from 3.5% (30 ProA) to 11.8% (1.5 GluA + 1.5 GluS). Our results are in agreement with Shafeek et al. (2012) findings that foliar application of glutamic acid resulted in significant increase yield of onion. The positive effect of tested amino acids on seed yield and its variables obtained in this study led suppose that they might play a role in synthesis of protein and nucleic acids, which might lead to improve plants productivity. These results are in line with Hildebrandt et al. (2015) statement that some amino acids might act as signaling molecules or may be precursors for the synthesis of secondary metabolites with signaling function.

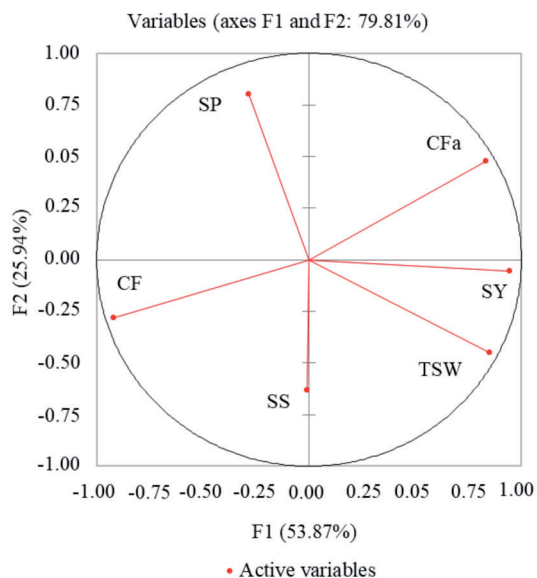
Scientific literature (Novickiene et al., 2010) indicates that the qualitative variables of seeds of winter rapeseed are largely determined by weather conditions during flowering and maturation of seeds. Some scientists propose that fat, protein, and glucosinolate contents in seeds are more determined by Genotype  $\times$  Environment interactions than by the applied cultivation technologies (Wielebski, 2009); other researchers have found no relationship between genotype and fat content (Karaaslan, 2008). Our studies show that the highest crude fat content and the lowest crude fiber content were found in winter rapeseed plants sprayed with L-glutamic acid solution in autumn and spring (1.5 GluA + 1.5 GluS).

Principal component analysis (PCA) was performed to evaluate the relationships between application of amino acids, elements of crop structure, and chemical content during the period 2015-2017. The first two components (PCs) were associated with eigenvalues greater than 1 and explained 53.87% and 25.94% of total variance (Figure 1). The first factor (PC1) was highly and positively correlated with seed yield, 1000-seed weight, and crude fat, and negatively with crude fiber, while the second factor (PC2) was highly and positively correlated with silique per plant and negatively with seeds per silique.

In Figure 2, the biplot relative to the PCA is shown. It shows that the elements of the yield structure as well as crude fat and fiber differed with different applications of amino acids, whereas all treatments were well separated in the PCA map.

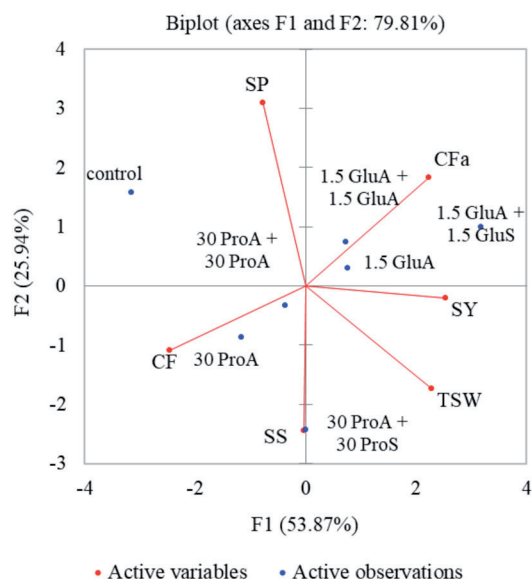
Principal component analysis showed that higher content of crude fat, seed yield, and 1000-seed weight were associated with the application of glutamic acid on winter rapeseed, while the amount of crude fiber in winter rapeseed was related to the application of proline.

**Figure 1. Factors loadings for the first two principal components (PC1 and PC2).**



CFa: Crude fat; SY: seed yield; TSW: 1000-seed weight; SS: seeds per silique; CF: crude fiber.

**Figure 2. Principal component analysis (PCA) for seed yield (SY), siliques per plant (SP), seeds per silique (SS), 1000-seed weight (TSW), crude fat (CFa), and crude fiber (CF) of winter rapeseed influenced by different concentrations and application times.**



Control: Without amino acids; 30 ProA: sprayed with 30 mM L<sup>-1</sup> L-proline in autumn at the stage of 4-5 leaves unfolded (BBCH 14-15); 30 ProA + 30 ProA: sprayed with 30 mM L<sup>-1</sup> L-proline in autumn at BBCH 14-15 and at the stage of 6-8 leaves unfolded (BBCH 16-18); 1.5 GluA: sprayed with 1.5 M L<sup>-1</sup> L-glutamic acid in autumn at BBCH 14-15; 1.5 GluA + 1.5 GluA: sprayed with 1.5 M L<sup>-1</sup> L-glutamic acid in autumn at BBCH 14-15 and BBCH 16-18; 30 ProA + 30 ProS: sprayed with 30 mM L<sup>-1</sup> L-proline in autumn at BBCH 14-15 and at the stage of yellow floral bud (BBCH 59); 1.5 GluA + 1.5 GluS: sprayed with 1.5 M L<sup>-1</sup> L-glutamic acid in autumn at BBCH 59.

## CONCLUSIONS

The study showed that the higher 1000-seed weight, seed yield and crude fat content in seeds of winter rapeseed was determined by spraying plants with 1.5 M L<sup>-1</sup> L-glutamic acid solution in autumn and spring, while the higher number of siliques per plant and crude fiber content was observed in the absence of amino acid application. The growing seasons had different effects on the investigated variables. In 2015, significantly higher 1000-seed weight and seed yield were observed, while a significantly higher number of seeds per silique was determined in 2017. No influence of growing season on the number of siliques per plant, crude fat, or crude fiber content was observed. A direct, strong and significant correlation between the 1000-seed weight and seed yield was determined. Principal component analysis results showed that 1000-seed weight, seed yield and crude fat content were associated with the application of L-glutamic acid, while crude fiber content was related to the application of L-proline.

## REFERENCES

- Bardgett, R.D., and Gibson, D.J. 2017. Plant ecological solutions to global food security. *Journal of Ecology* 105:859-864.
- Bulgari, R., Cocetta, G., Trivellini, A., Vernieri, P., and Ferrante, A. 2015. Biostimulants and crop responses: a review. *Biological Agriculture & Horticulture* 31:1-17. doi:10.1080/01448765.2014.964649.
- Committee for the Common Organization of Agricultural Markets. 2020. Oilseeds and protein crops market situation. European Commission Committee for the Common Organization of Agricultural Markets. Available at <https://circabc.europa.eu/sd/a/215a681a-5f50-4a4b-a953-e8fc6336819c/oilseeds-market%20situation.pdf> (accessed September 2020).
- Du Jardin, P. 2015. Plant biostimulants: definition, concept, main categories and regulation. *Scientia Horticulturae* 196:3-14. doi:10.1016/j.scienta.2015.09.021.
- Durrett, T.P., Benning, C., and Ohlrogge, J. 2008. Plant triacylglycerols as feedstocks for the production of biofuels. *Plant Journal* 54:593-607. doi:10.1111/j.1365-313X.2008.03442.x.

- Ertani, A., and Nardi, S. 2013. Review: long-term research activity on the biostimulant properties of natural origin compounds. *Acta Horticulturae* 1009:181-187. doi:10.17660/ActaHortic.2013.1009.22.
- Ettl, J., Bernhardt, H., Huber, G., Thuneke, K., Remmele, E., and Emberger, P. 2020. Evaluation of pure rapeseed oil as a renewable fuel for agricultural machinery based on emission characteristics and long-term operation behaviour of a fleet of 18 tractor. *SN Applied Sciences* 2:1711. doi:org/10.1007/s42452-020-03490-8.
- European Parliament and the Council of the European Union. 2015. Directive (EU) 2015/1513 of the European Parliament and of the Council of 9 September 2015 Amending Directive 98/70/EC Relating to the Quality of Petrol and Diesel Fuels and Amending Directive 2009/28/EC on the Promotion of the Use of Energy from Renewable Sources. Available at <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32015L1513&from> (accessed September 2020).
- Hildebrandt, T.M., Adriano, N.N., Araújo, W.L., and Braun, H.P. 2015. Amino acid catabolism in plants. *Molecular Plant* 8:1563-1579. doi:org/10.1016/j.molp.2015.09.0.
- IUSS Working Group WRB. 2015. World reference base for soil resources 2014, update 2015 International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106. FAO, Rome.
- Karaaslan, D. 2008. The effect of different nitrogen doses on seed yield, oil, protein and nutrient content of spring rape. *Pakistan Journal of Botany* 40:807-813.
- Kazan, K. 2015. Diverse roles of jasmonates and ethylene in abiotic stress tolerance. *Trends in Plant Science* 20:219-229.
- Khan, M.I.R., Fatma, M., Per, T.S., Anjum, N.A., and Khan, N.A. 2015. Salicylic acid induced abiotic stress tolerance and underlying mechanisms in plants. *Frontiers in Plant Science* 6:462. doi:10.3389/fpls.2015.00462.
- Klugmann-Radziemska, E., and Ciunel, K. 2013. Rapeseed pellet – a byproduct of biodiesel production – as an excellent renewable energy source. *Ecological Chemistry and Engineering Society* 18:109-119.
- Kocira, S. 2019. Effect of amino acid biostimulant on the yield and nutraceutical potential of soybean. *Chilean Journal of Agricultural Research* 79:17-25. doi:10.4067/S0718-58392019000100017.
- Koukounaras, A., Tsouvaltzis, P., and Siomos, A.S. 2013. Effect of root and foliar application of amino acids on the growth and yield of greenhouse tomato in different fertilization level. *Journal of Food Agriculture and Environment* 11:644-648. doi:10.1234/4.2013.4387.
- Kunicki, E., Grabowska, A., Sekara, A., and Wojciechowska, R. 2010. The effect of cultivar type, time of cultivation, and biostimulant treatment on the yield of spinach (*Spinacia oleracea* L.) *Folia Horticulturae* 22:9-13. doi:10.2478/fhort-2013-0153.
- Lotfi, R., Gharavi-Kouchebagh, P., and Khoshvaghti, H. 2015. Biochemical and physiological responses of *Brassica napus* plants to humic acid under water stress. *Russian Journal of Plant Physiology* 62:480-486. doi:10.1134/S1021443715040123.
- Mattner, S.W., Wite, D., Riches, D.A., Porter, I.J., and Arioli, T. 2013. The effect of kelp extract on seedling establishment of broccoli on contrasting soil types in southern Victoria, Australia. *Biological Agriculture & Horticulture* 29:258-270.
- Moreno-Hernández, J.M., Benítez-García, I., Mazonra-Manzano, M.A., Ramírez-Suárez, J.C., and Sánchez, E. 2020. Strategies for production, characterization and application of protein-based biostimulants in agriculture: A review. *Chilean Journal of Agricultural Research* 80:274-289. doi:10.4067/S0718-58392020000200274.
- Novickiene, L., Gaveliene, V., Miliuviene, L., Kazlauskienė, D., and Pakalniskytė, L. 2010. Comparison of winter oilseed rape varieties: cold acclimation, seed yield and quality. *Zemdirbyste-Agriculture* 97:77-86.
- Ozturk, H.H. 2014. Energy analysis for biodiesel production from rapeseed oil. *Energy Exploration & Exploitation* 32:1005-1031.
- Popko, M., Michalak, I., Wilk, R., Gramza, M., Chojnacka, K., and Górecki, H. 2018. Effect of the new plant growth biostimulants based on amino acids on yield and grain quality of winter wheat. *Molecules* 23:470. doi:10.3390/molecules23020470.
- Shafeek, M.R., Y.I. Helmy, Y.I., Shalaby M.A.F., and Omer N.M. 2012. Response of onion plants to foliar application of sources and levels of some amino acid under sandy soil conditions. *Journal of Applied Sciences Research* 8:5521-5527.
- Sierras, N., Botta, A., Staasing, L., Martínez, M.J., and Bru, R. 2016. Understanding the effect of amino acids based biostimulant by an enantiomeric analysis of their active principles and a proteomic profiling approach. *Acta Horticulturae* 1148:93-100. doi:10.17660/ActaHortic.2016.1148.11.
- Sorensen, M.T., and Danielsen, V. 2006. Effects of the plant growth regulator, chlormequat, on mammalian fertility. *International Journal of Andrology* 29:129-133.
- Sulek, M.W., Kulczycki, A., and Malysa, A. 2010. Assessment of lubricity of compositions of fuel oil with biocomponents derived from rape-seed. *Wear* 268:104-108.
- Szczepanek, M., Wilczewski, E., and Grzybowski, K. 2016. Response of winter oilseed rape (*Brassica napus* L.) on soil applied humus preparation and foliar potassium fertilizer. *Acta Scientiarum Polonorum Agricultura* 15:85-94.
- Tsouvaltzis, P., Koukounaras, A., and Siomos, A.S. 2014. Application of amino acids improves lettuce crop uniformity and inhibits nitrate accumulation induced by the supplemental inorganic nitrogen fertilization. *International Journal of Agriculture & Biology* 16:951-955. doi:13-300/2014/16-5-951-955.
- Wang, K., Lu, C., and Chang, S. 2011a. Evaluation of acute toxicity and teratogenic effects of plant growth regulators by *Daphnia magna* embryo assay. *Journal of Hazardous Materials* 190:520-528.



- Wang, X., Mathieu, A., Courède, P.H., Allirand, J.M., Jullien, A., de Reffye, P., et al. 2011b. Variability and regulation of the number of ovules, seeds and pods according to assimilate availability in winter oilseed rape (*Brassica napus* L.) Field Crops Research 122:60-69.
- Wielebski, F. 2009. Response of different types of winter oilseed rape varieties to crop production systems. Quality of seed yield. Rosliny Oleiste 30:91-101.
- Yasari, E., Patwardhan, A.M., Ghole, V.S., Omid, G.C., and Ahmad, A. 2008. Relationship of growth parameters and nutrients uptake with canola (*Brassica napus* L.) yield and yield contribution at different nutrients availability. Pakistan Journal of Biological Sciences 11:845-853. doi:10.3923/pjbs.2008.845.853.
- Zanetti, F., Vamerli, T., and Mosca, G. 2009. Yield and oil variability in modern varieties of high-erucic winter oilseed rape (*Brassica napus* L. var. *oleifera*) and Ethiopian mustard (*Brassica carinata* A. Braun) under reduced agricultural inputs. Industrial Crops and Products 30:265-270.
- Zhang, S.J., Chao, Y., Zhang, C.L., Cheng, J., Li, J., and Ma, N. 2010. Earthworms enhanced winter oilseed rape (*Brassica napus* L.) growth and nitrogen uptake. Agriculture, Ecosystems & Environment 139:463-468.
- Ziosi, V., Zandoli, R., and Di Nardo, A. 2013. Biological activity of different botanical extracts as evaluated by means of an array of *in vitro* and *in vivo* bioassays. Acta Horticulturae 1009:61-66. doi:10.17660/ActaHortic.2013.1009.5.