Journal of Elementology ISSN 1644-2296

Radkowski, A., Wolski, K., Radkowska, I., Burduk, R., Khachatryan, K., Bujak, H.
and Jelen, L. (2023)
'Variance analysis of an experimental study of effects of amino acids
and Fe chelate on turfgrass',
Journal of Elementology, 28(4), 1155-1174,
available: https://doi.org/10.5601/jelem.2023.28.3.3128

RECEIVED: 19 September 2023
ACCEPTED: 31 October 2023

ORIGINAL PAPER

Variance analysis of an experimental study of the effects of amino acids and Fe chelate on turfgrass

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Abstract

Fertilising turfgrass helps keep the turf green and in good aesthetic condition. Regular fertilisation makes turfgrass more resistant to adverse weather conditions, for example droughts, and gives it the characteristic dark green colour. Fertilisers also protect turfgrass from moss and various diseases. A visual assessment was carried out (general aspect, turf area, leaf colour and structure), in addition to which selected vegetation indices and the content of minerals were evaluated. Conducted at the Experimental Station of the University of Agriculture in Krakow, Poland, the research was carried out to assess the functional value of turfgrasses. An amino acid product sprayed at a dose of 2.0 dm^3 ha^{-1} and iron chelate at a dose of 130 g Fe ha^{-1} constituted experimental factors. The visual assessment of turfgrass characteristics was made on a 9-point scale. The mineral content of the plant material was also determined. During the growing period, the grass was mowed to a height of 6 cm. The combination of both products significantly increased the quality of turfgrass. The turfgrass in the plots treated with amino acids and Fe chelate applied together had the highest aesthetic and functional values. Satisfactory results were also obtained in plots where the amino acid product was applied on its own.

Keywords: growth stimulator, vegetation indices, leaf colour, leaf structure, ANOVA

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INTRODUCTION

The application of biostimulants containing free amino acids to grass species results in positive effects (Radkowski et al. 2020, 2021, Talar-Krasa et al. 2021). Products containing amino acids, having various functions in plants, stimulate many processes, such as root development, seed germination, photosynthesis, and chlorophyll formation (Radkowski, Radkowska 2018). Treatment of plants with amino acids not only stimulates their growth and development, but also strengthens their immune system and improves the absorption of nutrients, affecting their active transport (Kocira et al. 2015, Radkowski et al. 2018, Kocira, Kocira 2019). After the use of various amino acid-based biostimulants, increased plant growth and improved tolerance to biotic and abiotic stress are observed (Kocira et al. 2020). The improvement in plant resistance to stress factors as a result of biostimulant application is probably due to changes in enzymatic activity and increased synthesis of antioxidant compounds (Basak 2008, Calvo et al. 2014).

As in other plants, in turfgrass, amino acids are the building blocks of proteins that perform various metabolic functions. As metabolites and precursors, they are involved in the protection of turf plants against stress, in the biosynthesis of vitamin nucleotides and hormones, and are precursors of a wide variety of secondary compounds (Kandil et al. 2016). It can be concluded that amino acids as active catalysts or precursors are essentially involved in all regulatory and physiological aspects of turfgrass plant metabolism. According to the literature, 20 amino acids are considered essential for the proper development and functioning of turfgrass (Van Oosten et al. 2017) .

Biostimulants based on left-handed (natural) amino acids obtained by enzymatic hydrolysis have a significant impact on a number of turfgrass characteristics. In all types of grass mixtures, the visual assessment of turfgrass treated with biostimulants increases compared to untreated turfgrasses (Radkowski et al. 2020). To achieve favourable results, it is important to use such products in crucial stages of development for plant quality and yield (Kocira, Kocira 2019). Biostimulants are also recommended as an intervention method to be used in stressful conditions, such as frost, drought, hail, strong wind, and chemical contamination with pesticides. They can be used for better plant growth both before expected stress, during adverse conditions, and afterwards (Glińska et al. 2007). Biostimulants can be applied to the soil or to leaves, depending on their composition and expected results (Kunicki et al. 2010). However, they exert an effect only when they penetrate the plant tissue. This aspect should be taken into account when comparing their effects, as plant species can react differently to biostimulants that have different physicochemical characteristics (Kolomazník et al. 2012, Pecha et al. 2012). Thus, the effect of the same biostimulant may be different depending on both species and even varieties. In addition, it depends on environmental factors and on the method, dose and time of their application (Kunicki et al. 2010).

The second factor used in the experiment was Fe, an essential nutrient for all organisms (Zuo, Zhang 2011). Iron deficiency is common among many different crops (Sánchez-Alcalá et al. 2014). The Fe content of the soil is usually high, but it is largely related to the components of the soil (Mimmo et al. 2014, Bindraban et al. 2015). Insoluble Fe^{3+} compounds can be formed, especially at high pH and in aerobic soils, usually poor in the absorbable form of Fe^{2+} (Ye et al. 2015). Because plants usually take up Fe^{2+} from the soil, a deficiency of Fe in the soil entails its deficiency in plants (Kobayashi, Nishizawa 2012). In plants, Fe is involved in many physiological processes, including chlorophyll biosynthesis, respiration, and redox reactions (Mimmo et al. 2014, Ye et al. 2015, Zargar et al. 2015). Its deficiency leads to a decrease in the chlorophyll content, inducing chlorosis symptoms and negatively affecting the visual qualities of turfgrass. Therefore, the foliar application of products based on amino acids applied together with microelement fertilisers guarantees an immediate effect during unfavourable conditions or at critical stages of plant growth and development.

The purpose of the research was to assess the impact of the foliar application of iron chelate and amino acid products on the aesthetic value and functional value of turfgrasses planted with a mixture of grasses.

MATERIALS AND METHODS

The research was carried out at the Experimental Station of the University of Agriculture in Krakow (50°07′ N, 20°05′ E) from 2020 to 2022, and it was set up on degraded black earth developed from loess and classified as a very good soil suitable for wheat. Its chemical properties were as follows: pH_{KCl} – 6.5, N total – 2.52 g kg^{-1}, P – 65.23, K – 154.25, Mg – 42.30 mg kg^{-1}. The experiment was established according to the recommendations for soil treatment before planting a turfgrass. In the experiment, the functional value of the Super Wembleyka seed mixture was evaluated. The mixture included red fescue (*Festuca rubra* L.) var. Adio 18%, hard fescue (*Festuca trachyphylla* Hack.) var. Ridu 5%, tall fescue (*Festuca arundinacea* Schreb.) var. Escalante 15%, bluegrass (*Poa pratensis* L.) var. Evora 8%, permanent ryegrass (*Lolium perenne* L.) var. Boxer 34% and permanent ryegrass (*Lolium perenne* L.) var. Stadion 20%.

The seed mixture was sown on 10 m^2 plots, at a seeding amount of 2250 g 100 m^{-2} on 5 April 2020. In the year of sowing, the fertilisation of 650 g N 100 m^{-2}, 330 g P, 1245 g K 100 m^{-2} was used and the doses of 1900 g N 100 m^{-2}, 352 g P 100 m^{-2}, 1245 g K 100 m^{-2} were applied in the following growing seasons. 250 g N 100 m^{-2} was applied before sowing, then 200 g N 100 m^{-2} in May, and another 200 g N 100 m^{-2} in July. Nitrogen was applied in the form of ammonium nitrate (34% N), phosphorus as enriched superphosphate (17.4% P), and potassium in the form of potassium salt (49.8% K).

AGRO-SORB®, a plant growth stimulant containing amino acids applied at a dose of 2.0 dm^3 ha^{-1} was the first experimental factor. Plants sprayed with water (being a solvent for the biostimulant) served as a control. Appropriate amounts of amino acids were dissolved in water to prepare a dose of 300 dm^3 ha^{-1}.

As an amino acid product, AGRO-SORB® Folium is a growth stimulant with biologically active 18 free amino acids (L-alpha), obtained by enzyme hydrolysis. In its composition, it contains at least 9.3% (mole percentage) of biologically active free amino acids. These are: aspartic acid 0.450%, serine 0.321%, glutamic acid 1.814%, glycine 2.743%, histidine 0.208%, arginine 0.131%, threonine 0.323%, alanine 0.524%, proline 0.347%, cysteine 0.435%, tyrosine 0.174%, valine 0.551%, methionine 0.349%, lysine 0.661%, isoleucine 0.308%, leucine 0.180%, phenylalanine 0.218% and tryptophan 0.05% (data confirmed by chemical analysis). This biostimulant was applied to the leaves at the beginning of April, June and August, i.e. three times during the growing period. AGRO-SORB® Folium is produced by BIOPHARMACOTECH, a limited partnership, with its registered office in Częstochowa (Poland).

The second experimental component was a product called Fe-13 Microchelate™, applied to the leaves. Spray solutions were prepared by dissolving 1 dm^3 of Mikrochelat™ Fe-13 in such an amount of water as to obtain a spray volume of 300 dm^3 ha^{-1}. The 13% Fe chelate (EDTA chelate) contains 130 g Fe kg^{-1}. A dose of 130 g Fe ha^{-1} was used three times during the growing period, at the beginning of April, June and August. The Fe-13 Microchelate™ has been qualified by IUNG-PIB in Puławy for use in organic farming under the number NE/383/2017. It is produced by INTERMAG Ltd. in Olkusz (Poland).

During the growing period, the grass was mowed to a height of 6 cm. Mowing was carried out when the plants reached a height of 9 cm. The number and height of mowing were in accordance with the recommendations for non-sports grass mixtures (Domański 1998). During longer periods of drought (symptoms: soil at a depth of 3 cm dried, grass does not rise when pressed), watering in the amount of approximately 10 dm^3 m^{-2} was used systematically at 3-day intervals.

Turfgrass visual characteristics were rated on a 9-point scale (1 – bad quality, 9 – highly desirable quality) according to the assessment system applied to turfgrasses (Domański 1998). The following visual characteristics of lawns were evaluated: general aesthetic value, turf density, colour, and leaf structure.

The leaf greenness index (SPAD) was measured with the Minolta SPAD 502DL chlorophyllometer, the green leaf area index (LAI) with Delta-T

Sunscan System and the Normalized Difference Vegetation Index (NDVI) (Govaerts and Verhulst 2010, ‘Green Area Index. © Agriculture and Horticulture Development Board 2018. All rights reserved.’ 2022) was determined by the GreenSeeker device. The mineral content was determined using the Weenden method (AOAC, 2005).

Weather conditions

From 2020 to 2022, the weather conditions in the vicinity of the perennial ryegrass seed plantation were favourable for the growth and development of grass. Rainfall in these years was as follows: in 2020-605 mm, in 2021-807 mm, and in 2022-446 mm (Figure 1*a*). During the growing season from April to September, rainfall was: 385 mm in 2020, 633 mm in 2021, and 299 mm in 2022. The average air temperature in these years ranged at: 10.1°C (2020), 8.9°C (2021), and 9.0°C (2022). During the growing season (April-September), the average temperatures were: 16.0°C (2020), 15.3°C (2021), and 15.8°C (2022) – Figure 1*b*. Comparing these data, there are large differences

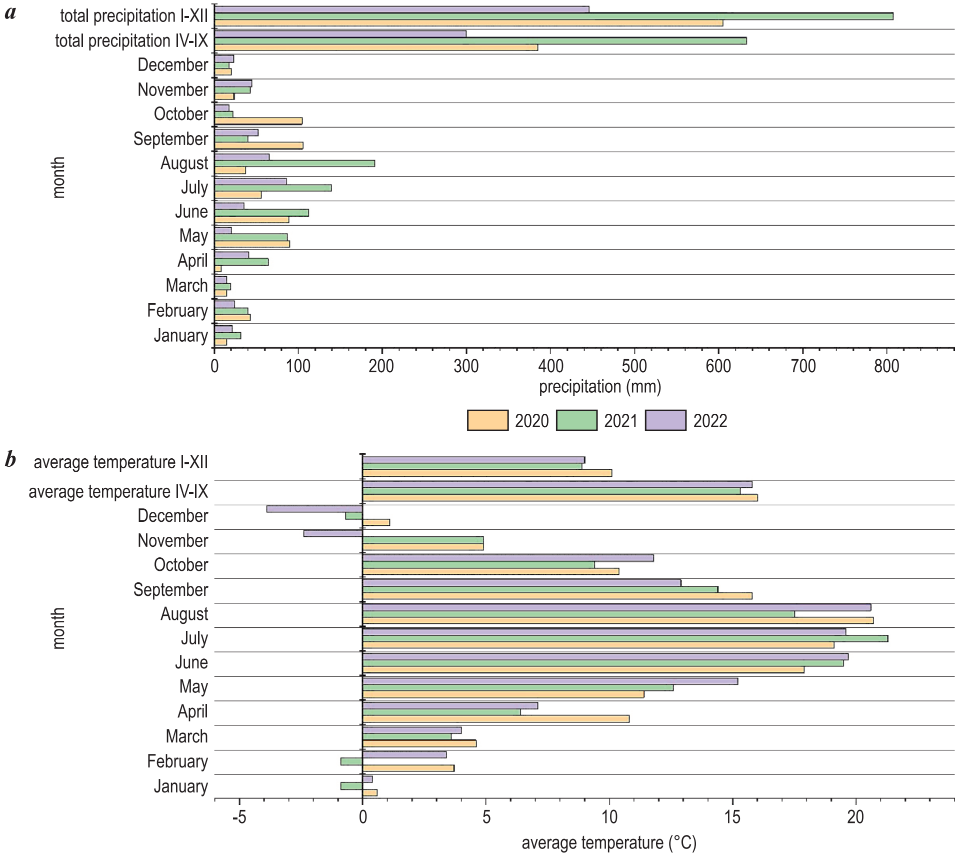

Fig. 1. Total precipitation (*a*) and mean air temperature (*b*) at the Experimental Station in Prusy, University of Agriculture in Kraków, in the years 2020-2022

in precipitation between the years, with 2021 standing out for its particularly high precipitation, which was almost double that of 2022.

Statistical analysis

The results were statistically processed using SAS Studio 3.8 software (820 SAS Campus Drive, Cary, NC, USA). First, the compatibility of the distribution of the individual variables with the normal distribution was checked. For this purpose, the Shapiro-Wilk normality test was used. For almost all variables, excluding Na and Mg, it was assumed that their distribution was compatible with the normal distribution, as marked in Table 1. Then, an analysis of variance (ANOVA) was performed to verify the null hypotheses of a lack of treatment and the effect of the annual season on the seven variables, independently for each one. In this analysis, the homogeneity of variance was first tested using the Levine's test. In all analysed cases, the Levine's test indicated the homogeneity of variance. Tables 3-8 also show the Fisher's least significant differences (LSDs) values at a significance level of α=0.05. Furthermore, the relationships between variables were estimated using the Pearson's linear correlation coefficients.

RESULTS

The results of the analysis of variance indicated a statistically significant effect of treatment combinations and annual seasons on the visual assessment of plants (overall appearance, turf density, leaf colour, and leaf structure, i.e., leaf fineness) and on the vegetation index of turfgrasses. No statistically significant impact on the macro- and micronutrient content was observed, except for iron (Table 1). The interaction between treatment combinations and annual seasons was statistically significant only for visual characteristics.

According to the results (Table 2), the application of both products positively affected visual characteristics. Depending on the treatment variant and the annual season, the appearance ratings of the turfgrass ranged from 4.75 to 8.00 (Table 2). Each treatment combination significantly affected the aesthetic value of the turfgrasses. Considering the annual seasons, the highest values were recorded in autumn, slightly lower in spring, and the lowest in summer. According to Morris et al. (Morris et al. 2022), the National Turfgrass Evaluation Program – NTEP ('National Turfgrass Evaluation Program – WELCOME' 2022) and Xiang (Xiang, Fry 2019), significant variations occur when the differences between average ratings are at least 0.5 points.

Another characteristic evaluated was the soil cover by leaf blades during the growing period, also called turf density. The more leaf blades cover the

Table 1

Mean squares from the two-way analysis of variance for turfgrass characteristics

Source of variation	Season	Treatment	Treatment x Season	Residual
Degrees of freedom	2	3	6	36
Overall appearance	54.0156 *	23.1406 *	7.6406*	0.0525
Turf density	33.9375*	28.4322*	4.5516*	0.1491
Leaf colour	45.2244*	27.2430*	5.3315*	0.0888
Leaf structure (fineness)	45.8125*	22.1822*	8.7291*	0.1809
LAI	0.4810*	0.0783*	0.0033	0.0036
NDVI	0.0108*	0.0259*	0.0001	0.0001
SPAD	279.0370*	87.3275*	1.0708	0.7909
P	1.0070*	1.4383*	0.1427	0.1155
K	84.9736*	5.2659	7.9172	6.6530
Na**	0.0010	0.0070	0.0064	0.0011
Ca	0.7121*	0.3544*	0.7589*	0.1230
Mg**	1.0906*	1.0073*	0.4532*	0.1253
Mn	353.0978	1100.18*	989.2306*	197.6791
Fe	118976*	12329*	633.21*	44.4631
Zn	1107.40*	248.0322	699.7325*	108.6209
Cu	17.7870*	5.1307*	2.9775	1.4811

* $P<0.05$, ** reject the null hypothesis of normal distribution

soil, the higher the rating. Of all characteristics, the largest variation coefficient was for turf density ratings during summer (V = 21.61%). Depending on the treatment variant, they ranged between 4.5 and 8.0 (Table 2). Treatment with iron chelate alone significantly affected the assessment of turfgrasses significantly ($p \leq 0.05$). Ratings on plots treated with amino acids and on those with both products used together (variants I and III) were significantly higher ($p \leq 0.05$) than for control plants.

The highest leaf colour ratings were assigned to plants in plots with amino acids and iron chelate (Table 3). Compared to control plants, the turfgrass ratings treated with both products were on average 2 points higher. Similarly, the structure of the leaf in spring and summer was rated highest in plots treated with the combination of amino acids and iron chelate. It was, on average, 2.7 higher than for control plants.

The Leaf Area Index (LAI) values between treatments and annual seasons are presented in Table 4. No significant differences were found between the effects of the treatment combinations in spring and summer. A significantly higher LAI value was observed only in the autumn for plants to which both products were applied (variant III).

Table 2

The effect of amino acids and Fe chelate on the overall appearance of turfgrass and the turf density ratings on a 9-point scale (mean values ± standard deviation)

Treatment	Years	Overall appearance			Turf density		
		spring	summer	autumn	spring	summer	autumn
Control	2020	5.450±0.180	4.780±0.178	7.880±0.178	6.750±0.520	4.650±0.210	7.880±0.240
	2021	5.430±0.230	4.650±0.235	7.730±0.228	6.300±0.450	4.350±0.170	7.740±0.158
	2022	5.250±0.120	4.820±0.282	7.640±0.284	6.450±0.510	4.500±0.280	7.630±0.245
	2020-2022	5.375*c*[#]±0.226	4.750*c*±0.261	7.750*b*±0.261	6.500*b*±0.522	4.500*d*±0.255	7.750*c*±0.261
Variant I (AA)	2020	7.650±0.248	6.250±0.325	8.120±0.240	8.330±0.250	6.600±0.457	8.200±0.420
	2021	7.820±0.158	6.000±0.242	8.140±0.265	8.150±0.214	6.450±0.580	8.000±0.542
	2022	7.780±0.230	6.750±0.235	7.740±0.324	7.900±0.352	6.450±0.620	7.800±0.523
	2020-2022	7.750*b*±0.261	6.000*b*±0.254	8.000*a*±0.258	8.125*a*±0.226	6.500*c*±0.522	8.000*b*±0.254
Variant II (Fe)	2020	7.750±0.320	5.200±0.456	8.000±0.120	7.900±0.352	7.200±1.240	7.900±0.152
	2021	7.480±0.214	5.000±0.453	8.100±0.220	8.100±0.206	7.180±1.320	8.120±0.234
	2022	7.650±0.250	4.800±0.577	7.900±0.155	8.000±0.420	7.000±1.108	8.000±0.250
	2020-2022	7.625*b*± 0.226	5.000*c*±0.522	8.000*a*± 0.258	8.000*a*±0.142	7.125*b*±1.002	8.000*b*± 0.320
Variant III (Fe+AA)	2020	8.000±0.215	7.700±0.425	8.000±0.574	8.300±0.528	7.900±0.420	8.900±0.310
	2021	8.100±0.158	7.550±0.320	8.150±0.652	8.200±0.326	8.100±0.324	8.850±0.354
	2022	7.900±0.145	7.600±0.250	7.850±0.412	8.250±0.288	8.000±0.260	8.875±0.250
	2020-2022	8.000*a*±0.230	7.625*a*±0.226	8.000*a*±0.437	8.250*a*±0.261	8.000*a*±0.232	8.875*a*±0.226
LSD$_{0.05}$		0.169	0.257	0.107	0.256	0.465	0.142
Mean± SD		7.187±1.084	5.843±1.181	7.937±0.167	7.718±0.778	6.531±1.411	8.156±0.463
Variation coefficient (%)		15.09	20.21	1.10	10.08	21.61	5.76

[#] *a*, *b*, *c*, *d* – means in columns marked with different letters differ significantly ($P \leq 0.05$), AA – amino acids

Table 3

The effect of amino acids and Fe chelate on leaf colour and leaf structure ratings on a 9-point scale (mean values ± standard deviation)

Treatment	Years	Leaf colour			Leaf structure		
		spring	summer	autumn	spring	summer	autumn
Control	2020	5.190±0.324	5.100±0.124	7.300±0.450	4.600±0.354	4.690±0.321	8.100±0.142
	2021	5.060±0.335	5.000±0.230	7.200±0.520	4.500±0.408	4.540±0.250	8.000±0.212
	2022	5.125±0.250	4.900±0.245	7.250±0.500	4.400±0.257	4.650± 0.423	7.900±0.320
	2020-2022	5.125*c*[#]±0.226	5.000*d*±0.326	7.250*b*±0.452	4.500*d*±0.369	4.625*c*±0.226	8.000*a*±0.215
Variant I (AA)	2020	8.200±0.322	5.900±0.215	8.100±0.312	7.400±0.425	6.100±0.356	8.150±0.520
	2021	8.110±0.250	5.840±0.356	7.900±0.263	7.300±0.348	5.900±0.362	8.150±0.432
	2022	8.070±0.145	5.880±0.250	8.000±0.241	7.420±0.478	6.000±0.408	8.100±0.478
	2020-2022	8.125*a*±0.226	5.875*b*±0.226	8.000*a*±0.124	7.375*b*±0.433	6.000*b*±0.369	8.125*a*±0.433
Variant II (Fe)	2020	7.250±0.360	5.600±0.626	8.100±0.472	6.600±0.547	6.300±0.742	7.100±0.215
	2021	7.250±0.422	5.400±0.577	7.900±0.332	6.400±0.623	6.200±0.635	6.900±0.334
	2022	7.125±0.250	5.500±0.456	8.000±0.251	6.500±0.707	6.250±0.457	7.000±0.352
	2020-2022	7.208*b*±0.396	5.500*c*±0.522	8.000*a*±0.254	6.500*c*±0.639	6.250*ab*±0.866	7.000*b*±0.265
Variant III (Fe+AA)	2020	8.300±0.323	7.300±0.234	8.100±0.638	8.100±0.543	6.600±0.689	8.100±0.253
	2021	8.200±0.245	7.250±0.328	7.900±0.627	8.000±0.357	6.500±0.521	8.000±0.364
	2022	8.250±0.288	7.200±0.500	8.000±0.520	7.900±0.246	6.400±0.707	7.900±0.358
	2020-2022	8.250*a*±0.261	7.250*a*±0.452	8.000*a*±0.452	8.000*a*±0.359	6.500*a*±0.639	8.000*a*±0.263
LSD$_{0.05}$		0.235	0.299	0.186	0.352	0.477	0.178
Mean± SD		7.177±1.294	5.906±0.914	7.812±0.394	6.593±1.397	5.843±0.923	7.781±0.504
Variation coefficient (%)		18.03	15.48	5.04	21.19	15.80	6.48

[#] *a*, *b*, *c*, *d* – means in columns marked with different letters differ significantly ($P \leq 0.05$), AA – amino acids

Table 4

The effect of amino acids and Fe chelate on turfgrass LAI and NDVI values (mean values ± standard deviation)

Treatment	Years	LAI			NDVI		
		spring	summer	autumn	spring	summer	autumn
Control	2020	1.041±0.095	0.907±0.068	1.076±0.081	0.775±0.002	0.756±0.002	0.783±0.002
	2021	1.077±0.042	0.905±0.071	1.060±0.081	0.790±0.000	0.762±0.000	0.790±0.000
	2022	1.027±0.053	0.887±0.066	1.047±0.086	0.742±0.009	0.747±0.005	0.762±0.005
	2020-2022	1.048*c*±0.065	0.899*c*±0.062	1.061*c*±0.076	0.769*d*±0.021	0.755*d*±0.007	0.778*d*±0.012
Variant I (AA)	2020	1.118±0.053	0.963±0.054	1.158±0.062	0.817±0.003	0.793±0.002	0.830±0.010
	2021	1.125±0.054	0.957±0.057	1.140±0.060	0.835±0.005	0.802±0.005	0.840±0.008
	2022	1.102±0.053	0.940±0.052	1.125±0.065	0.807±0.005	0.782±0.005	0.810±0.008
	2020-2022	1.115*ab*±0.049	0.953*ab*±0.050	1.141*b*±0.058	0.820*b*±0.012	0.792*b*±0.009	0.826*b*±0.015
Variant II (Fe)	2020	1.082±0.088	0.927±0.069	1.104±0.079	0.798±0.001	0.778±0.001	0.807±0.001
	2021	1.090±0.092	0.922±0.069	1.085±0.080	0.812±0.005	0.787±0.005	0.817±0.005
	2022	1.067±0.088	0.905±0.070	1.075±0.080	0.790±0.000	0.770±0.000	0.787±0.005
	2020-2022	1.079*bc*±0.081	0.918*bc*±0.064	1.088*bc*±0.073	0.800*c*±0.010	0.778*c*±0.007	0.804*c*±0.013
Variant III (Fe+AA)	2020	1.140±0.031	0.992±0.035	1.225±0.054	0.833±0.003	0.812±0.003	0.849±0.002
	2021	1.147±0.033	0.987±0.035	1.205±0.055	0.850±0.000	0.820±0.003	0.857±0.005
	2022	1.125±0.031	0.967±0.035	1.195±0.055	0.822±0.005	0.802±0.005	0.827±0.005
	2020-2022	1.137*a*±0.030	0.982*a*±0.034	1.208*a*±0.051	0.835*a*±0.012	0.811*a*±0.008	0.844*a*±0.013
$LSD_{0.05}$		0.049	0.044	0.054	0.012	0.006	0.011
Mean± SD		1.095±0.067	0.938±0.061	1.124±0.085	0.806±0.028	0.784±0.022	0.813±0.028
Variation coefficient (%)		6.14	6.57	7.58	3.55	2.83	3.49

[#] *a*, *b*, *c*, *d* – means in columns marked with different letters differ significantly ($P \leq 0.05$),AA – amino acids

The average Normalized Difference Vegetation Index (NDVI) value in consecutive seasons was 0.806 in the spring, 0.785 in the summer and 0.813 in the autumn (Table 4). Its significant variations across treatments were found for all treatment combinations. The values of plants treated with amino acids and with both products (variants I and III) were significantly higher ($p \leq 0.05$) than in the control.

The leaf greenness index (SPAD) varied across the annual seasons, ranging from 33.947 to 41.930 (Table 5). Statistically significant differences were found between treatment combinations. On the treated plots, the SPAD values were higher than on the control ones. The application of amino acids on their own and of both products together resulted in increased SPAD values.

The results presented in Table 6 indicate large differences in the content of macronutrients in plants. The highest variation coefficient was recorded for Na (V = 36.42%), and the smallest one – for K (V = 7.12%). When applied to turfgrasses, Fe chelate significantly increased the content of P and Mg. Their increased content was also recorded when both products were applied together, although the increase was not significant.

Table 7 presents a weighted average of the micronutrient content. It depended on the treatment and varied in the following ranges (mg kg^{-1} DM): 11.429-12.351 for Cu, 149.243-160.785 for Mn, 314.635-357.615 for Fe, 69.472-75.747 for Zn. The highest variability in micronutrient content was

Table 5

The effect of amino acids and Fe chelate on SPAD values of the turfgrass mixture (mean values ± standard deviation)

Treatment	Years	SPAD		
		spring	summer	autumn
Control	2020	34.570±0.905	33.947±0.888	37.747±0.766
	2021	35.015±0.717	34.630±0.905	38.507±0.784
	2022	33.072±0.549	31.100±0.866	36.802±0.747
	2020-2022	34.219*c*[#]±1.095	33.892*d*±1.034	37.685*d*±1.005
Variant I (AA)	2020	36.310±0.171	36.372±0.172	40.890±0.540
	2021	37.040±0.171	37.102±0.172	41.715±0.553
	2022	35.400±0.163	35.460±0.167	39.867±0.523
	2020-2022	36.250*b*±0.717	36.311*b*±0.718	40.824*b*±0.928
Variant II (Fe)	2020	35.867±0.643	35.030±0.424	39.527±0.708
	2021	36.592±0.656	35.737±0.436	40.325±0.720
	2022	34.972±0.623	34.157±0.415	38.540±0.686
	2020-2022	35.810*b*±0.903	34.975*c*±0.776	39.464*c*±0.994
Variant III (Fe+AA)	2020	37.750±0.276	37.297±0.241	41.930±0.323
	2021	38.510±0.282	38.050±0.250	42.777±0.328
	2022	36.767±0.201	36.370±0.236	40.885±0.313
	2020-2022	37.675*a*±0.780	37.239*a*±0.750	41.864*a*±0.859
LSD$_{0.05}$		0.728	0.628	0.780
Mean± SD		35.988±1.512	35.604±1.517	39.959±1.828
Variation coefficient (%)		4.20	4.26	4.57

[#] *a*, *b*, *c*, *d* – means in columns marked with different letters differ significantly ($P \leq 0.05$), AA – amino acids

Table 6

Effect of amino acids and Fe chelate on macronutrient content (g kg^{-1} DM) of the turfgrass mixture (mean values + standard deviations)

Treatment (dm^3 ha^{-1})	Years	Macronutrient content (g kg^{-1} DM)				
		P	K	Ca	Mg	Na
Control	2020	2.107±0.281	39.004±3.240	2.978±0.344	1.751±0.242	0.110±0.044
	2021	2.128±0.284	39.403±3.271	3.010±0.346	1.770±0.245	0.110±0.044
	2022	2.078±0.277	38.517±3.198	2.944±0.338	1.738±0.241	0.110±0.044
	2020-2022	2.104*c*[#]±0.273	38.975*a*±3.164	2.975*b*±0.334	1.750*c*±0.234	0.110*ab*±0.044
Variant I (AA)	2020	2.440±0.442	39.151±2.487	2.957±0.460	1.900±0.507	0.095±0.023
	2021	2.465±0.447	39.554±2.511	2.986±0.466	1.918±0.512	0.096±0.025
	2022	2.410±0.437	38.667±2.455	2.920±0.455	1.875±0.501	0.095±0.023
	2020-2022	2.439*ab*±0.430	39.124*a*±2.441	2.958*a*±0.448	1.897*bc*±0.492	0.095*bc*±0.023
Variant II (Fe)	2020	2.582±0.338	38.814±2.688	3.170±0.413	2.154±0.422	0.091±0.030
	2021	2.608±0.341	39.814±2.714	3.203±0.418	2.177±0.424	0.093±0.031
	2022	2.550±0.333	38.333±2.656	3.132±0.407	2.129±0.416	0.091±0.030
	2020-2022	2.580*a*±0.329	38.786*a*±2.634	3.168*b*±0.402	2.153*a*±0.409	0.092*c*±0.029
Variant III (Fe+AA)	2020	2.405±0.398	39.705±2.962	3.084±0.412	1.969±0.393	0.123±0.047
	2021	2.429±0.403	40.110±2.990	3.118±0.416	1.989±0.397	0.125±0.047
	2022	2.371±0.403	39.210±2.923	3.048±0.406	1.945±0.388	0.120±0.045
	2020-2022	2.401*b*±0.386	39.675*a*±2.897	3.083*b*±0.400	1.967*b*±0.382	0.122*a*±0.045
LSD$_{0.05}$		0.167	1.303	0.337	0.182	0.017
Mean± SD		2.381±0.396	39.140±2.787	3.046±0.403	1.942±0.413	0.105±0.038
Variation coefficient (%)		16.63	7.12	13.24	21.29	36.42

[#] *a*, *b*, *c* – means in columns marked with different letters differ significantly ($P \leq 0.05$), AA – amino acids

Table 7

The effect of amino acids and Fe chelate on the micronutrient content (mg kg^{-1} DM) of the turfgrass mixture (mean values + standard deviations)

Treatment (dm^3 ha^{-1})	Years	Micronutrient content (mg kg^{-1}DM)			
		Cu	Mn	Fe	Zn
Control	2020	12.351±1.352	149.243±11.133	314.635±50.994	73.685±8.875
	2021	12.477±1.364	150.770±11.245	317.854±51.516	74.439±8.963
	2022	12.199±1.334	147.393±10.994	310.735±50.361	72.771±8.764
	2020-2022	12.342*a*[#]±1.316	149.135*c*±10.892	314.408*c*±49.570	73.632*ab*±8.638
Variant I (AA)	2020	11.908±1.464	160.785±10.157	347.625±39.863	75.747±15.793
	2021	12.030±1.478	162.785±10.264	351.625±40.271	76.522±15.954
	2022	11.760±1.444	158.792±10.032	343.315±39.369	74.808±15.595
	2020-2022	11.900*ab*±1.424	160.669*a*±9.972	347.373*ab*±38.819	75.692*a*±15.340
Variant II (Fe)	2020	11.960±1.662	159.734±17.764	335.170±42.023	69.472±14.704
	2021	12.084±1.680	161.366±17.947	338.600±42.454	70.183±14.855
	2022	11.813±1.640	157.753±17.544	331.016±41.502	68.610±14.521
	2020-2022	11.952*ab*±1.617	159.618*ab*±17.303	334.929*b*±40.898	69.421*b*±14.283
Variant III (Fe+AA)	2020	11.429±0.876	152.870±21.012	357.615±38.926	73.530±9.126
	2021	11.547±0.885	154.433±21.227	361.270±39.324	74.284±9.220
	2022	11.288±0.866	150.975±20.752	353.180±38.445	72.620±9.012
	2020-2022	11.421*b*±0.857	152.759*bc*±20.438	357.355*a*±37.921	73.478*ab*±8.882
LSD$_{0.05}$		0.621	7.125	19.598	5.673
Mean±SD		11.904±1.359	155.54±15.874	338.51±44.612	73.056±12.260
Variation coefficient (%)		11.42	10.20	13.17	16.78

[#] *a*, *b*, *c* – means in columns marked with different letters differ significantly ($P \leq 0.05$), AA – amino acids

recorded for Zn (V =16.78%), and the smallest for Mn (V=10.20%). Different combinations of treatments did not significantly affect the content of micronutrients, except for Fe.

Figure 2 visualises the strength of relationships measured by the Pearson correlation coefficient. The colour-coding of the cells represents the Pearson correlation coefficient value. If the cell colour is red, it is said to be a positive correlation between two variables. If the cell colour is white, there is no correlation between the two variables. If the cell colour is blue, it is said to be a negative correlation between the two variables. It is evident that the variables such as Fe & overall, Fe & density, Fe & colour and Fe & structure have quite strong positive correlations. An analysis of the entire correlation heatmap leads to the following conclusions: statistically significant positive correlation coefficients were found between all visual characteristics on the one hand and the vegetation index and Fe content on the other; negative correlation coefficients between visual characteristics and K and Cu content are noticeable.

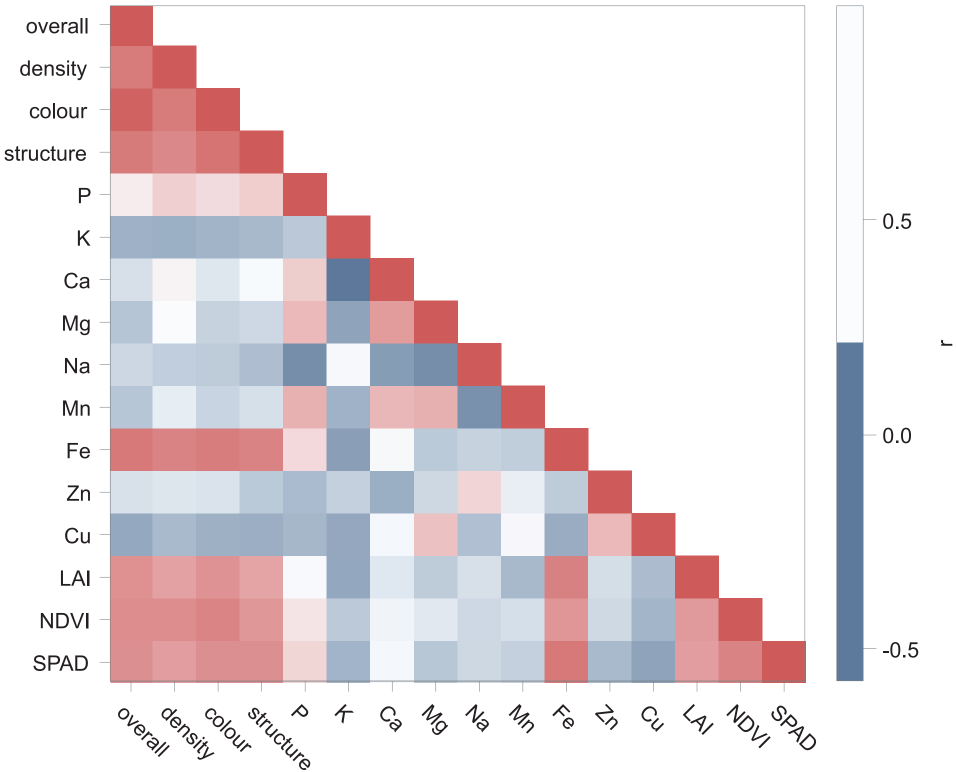

Fig. 2. Heatmap for the Pearson's linear correlation coefficients between observed characteristics

DISCUSSION

The investigation of biological systems and nature is exceedingly intricate since quantifying the effects of numerous factors (such as temperature, pressure, humidity, radiation, and pollutants in the air and soil) is unfeasible. Research on many crops has shown that the foliar application of amino acid-based biostimulants improves the absorption of nutrients from the soil, and has a favourable effect on the primary and secondary metabolism of plants. Field experiments have shown that amino acids not only improve plant nutritional absorption (Zodape et al. 2011, Nardi et al. 2016, Szczepanek, Grzybowski 2016), but also support germination (Carvalho et al. 2013), increase vegetative growth (Zhang, Hamauzu n.d.), increase chlorophyll content (Thirumaran et al. 2009), and improve yield and plant quality (Kowalczyk et al. 2008, Kunicki et al. 2010, Shehata et al. 2011).

Kauffman et al. (2007) reported the beneficial effect of applying a leaf biostimulator based on amino acids to perennial grass under heat stress conditions and pointed out that amino acids were beneficial under abiotic stress conditions. The plants were tested in three separate experiments in a growth chamber using optimal growth conditions and high air temperature pressure (20, 28, 36°C).

Mahdavi et al. (2017) obtained better results when evaluating the yield of perennial ryegrass treated with two biostimulants consisting of s-abscisic acid (s-ABA) and glycine betaine (GB). The grass clippings from the GB treated grass had significantly higher N than any other biostimulator or nontreated control. Furthermore, the GB-treated plot clippings had significantly higher S, K, and Cl but lower Zn. The K of the cuts of the s-ABA-treated turfgrass was also significantly higher than that of the untreated control. The average performance ratings of s-ABA and GB, s-ABA and GB, are significantly higher than those of the nontreated control, emphasising the value of these biostimulants in reducing drought stress.

Our research shows that only P, Mg, and Fe concentrations are higher, but each species may react differently to the amino acids applied. De Luca and others (2020), in the testing of three biostimulators, also obtained high quality ryegrass grass, especially when biostimulators including amino acids, polysaccharides, nitrogen, and microelements were used. This turf was more stable, darker in colour, and improved in growth and pruning compared to the control without treatment. The authors noted that the improvements observed in grass grazing of grass treated with this biostimulator could be related to the combination of all compounds in the mixture. The tested biostimulants also increase turf quality under nutritional pressure. In fact, the study included nutritional stress to demonstrate that biostimulators could temporarily replace the role of fertilisation and thus reduce environmental impacts. The use of biostimulators with amino acids and other substances has shown that it is possible to reduce the amount of mineral fertilisers. We also showed that the combination of amino acids and iron chelates had a better effect on turfgrass quality. Therefore, further research is needed to confirm these preliminary findings. Identify the optimal composition with other minerals to benefit both the quality of the turfgrass and the environmental benefits.

Noroozlo et al. (2019) showed the beneficial effect of amino acids on the growth of sweet basil. The fresh and dry weight of the leaf, the leaf area, the SPAD value of the leaf, and the chloride content of the leaf are improved by using glycine or glutamine in the leaf compared to control plants. The application of foliar amino acids increased the contents of nitrogen, magnesium, iron, and zinc. The results indicate that the application of a medium with low concentrations of glutamine or glycine to the leaf can improve basil growth Matysiak et al. (2020). It was shown that Gly had a negative impact on the fresh weight of stressed corn plants. The authors clearly demonstrated that the use of L-arginine has a positive impact on the growth and development of corn plants under stress conditions. Glycine does not have biological stimulation properties but may be beneficial at higher concentrations. These studies show that L-Arg is useful in preserving corn from dynamic temperature stressors.

Biostimulators promote the synthesis and accumulation of plant chemicals and increase plant tolerance to abiotic stress (Samson et al. 2004, Colla et al. 2012, Lucini et al. 2015, Xu, Leskovar 2015). Increased crop productivity under optimal conditions through biostimulators can be linked to several direct and indirect interaction mechanisms, including the stimulation of enzyme activities involved in Ca and N metabolism, Krebs cycles, and glycolysis. Biostimulants, particularly asogens and gibberellins, induce mechanisms similar to hormone activity and improve the nutritional status of treated plants by regulating the development of the root system (Colla et al. 2015, Colla, Cardarelli, et al. 2017, Colla, Hoagland et al. 2017).

According to Yousfi et al. (2021), the use of biostimulants improved the microbial activity, organic matter and enzymatic activity of two types of soil, sandy and sandy loam soils. Along with the use of biostimulants, the content of calcium, potassium, magnesium and phosphorus in the soil increased. Visconti et al. (2020), in turn, found that compost supplementation in combination with two biostimulators produced the best results in terms of plant growth and nutrient uptake.

The main feature that determines the aesthetic value of turfgrass is its appearance and aesthetic value (Turgeon 2012). Biobiotic and abiotic stress affects plant growth and development and its visual aspect, while the use of bioproducts supports plant physiological processes (Gugaa et al. 2017) and can compensate for this effect. According to Brown and Saa (2015), Du Jardin (2015) and De Pascale (2015) and De Pascale et al., (2017), biostimulants promote growth and/or reduce adverse effects of stress factors such as salinity, drought, temperature fluctuations, and pathogens to improve the overall condition of plants. They can trigger mechanisms for fast damage repair (Cavo et al., 2014). Biostimulants improve plant productivity by influencing plant communication pathways and reducing stress-related reactions (Brown, Saa 2015, Posmyk, Szafraska 2016). Unlike bioregulators and hormones, biostimulants improve plant metabolism without directly altering the natural pathway (Brown, Saa 2015). In addition to the economic aspect, one of the main factors affecting the increased use of biostimulants is environmental awareness and increased environmental regulations. Innovative solutions for plant production are being introduced to limit the use of chemical plant protection products and mineral fertilisers in favour of substances of natural origin. According to Van Dyke et al. (2008), it is particularly important to use biostimulants to maintain the high quality of the sports surface. As a result, we can reduce or completely eliminate other substances that have a negative impact on the natural environment.

Studying the impact of commercial biostimulants on turf quality, (Mueller, Kussow 2005) found a significant increase in the assessment values of visual parameters such as colour and turf density, but did not analyse general appearance. On the other hand, Daneshvar et al. (2014) attributed high evaluations of the characteristics of perennial ryegrass (*Lolium perenne* L.) characteristics to plant growth hormones in the substances applied in the experiment.

The main features that determine the aesthetic value of turfgrass are its appearance or aesthetic value (Turgeon 2012). Bio- and abiotic stress has a negative impact on plant growth, development, and visual properties (Raja et al. 2019), and bioproducts support plant physiological processes (Gagawa et al. 2017), and can compensate for these effects. According to Brown and Saa (2015), Jardin (2015), and Pascal (2015), biostimulants improve the general state of plants by stimulating growth and reducing the harmful effects of stress factors such as salinity, drought, temperature fluctuations, and pathogens. They can trigger rapid repair mechanisms (Carva et al. 2014). Biostimulators increase plant productivity by changing plant communication pathways, reducing negative stress responses (Brown, Saa 2015, Posinski, Szafroska 2016). The main point is that, unlike biological regulators and hormones, biostimulants can improve plant metabolic processes without directly changing natural pathways (Brown, Saa 2015). In addition to economic considerations, an important factor that influences the increase in the use of biostimulators is the growing environmental awareness and more stringent environmental regulations. Innovative solutions have been introduced to limit the use of chemicals and mineral fertilizers for plant protection in plant production to favour natural substances. According to Van Dyke et al. (2008), biostimulants are particularly important in the maintenance of high-quality sports grasses. Therefore, we can reduce or completely eliminate the concentrations of other substances that have harmful effects on the natural environment (Figure 3).

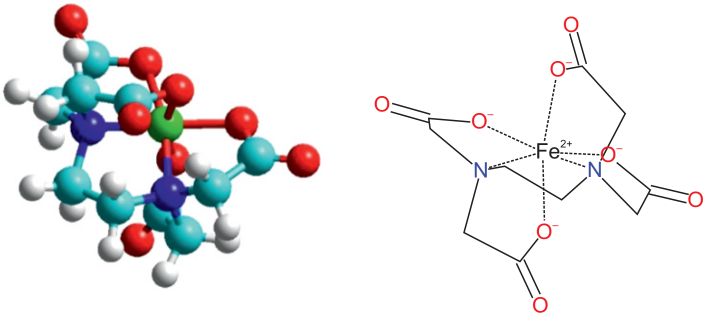

Fig 3. Structure of the EDTA-Fe(II) chelate (ball-and-stick model on the left, and structural formula on the right)

In addition to preventing oxidation of iron (II) to iron (III) ions, Fe(II)-EDTA complexes also facilitate the transportation of various elements necessary for plant development. Li et al. (2016) suggested that nitrogen oxides were well absorbed and that NO binding in Fe(II)EDTA-NO was biologically reduced to nitrogen. Subsequently, this process is reduced to primary compounds in the form of oxidised compounds by iron-reducing bacteria. Other researchers (Han et al., 2016) have shown that the ammonia-(FeII) EDTA complex can absorb exhaust gases (SO_2 and NO). Zhang et al. (2022) showed that the taste and nutritional value of tomatoes were improved by spraying the leaves of Fe-EDTA chelate fertilisers.

Turfgrass becomes yellow in summer due to a lack of N fertilisers. Fe addition can provide the desired dark green colour without causing excessive growth after N fertilization (SL21/LH014: General recommendations for flora fertilization of tertiary plants in Florida soils). Slow growth rate is an important factor to be taken into account in the assessment of grasslands (Wolski et al., 2021). Carrow et al. (2001) showed positive effects of using chelated Fe (EDTA, DTPA and EDDHA), but unchelated Fe oxidises quickly and cannot be absorbed into soil solutions (Shaddox et al. 2016). Consequently, it is not recommended to use untreated Fe granules unless Fe is treated in one of the aforementioned forms. However, the effects of Fe greening are often temporary and do not replace N fertilisers. Therefore, Fe cannot replace N because the latter provides the building material necessary for grasses to grow and achieve the desired appearance (Shaddox, n.d.). To achieve the expected visual effects, fertiliser can be combined with these two chemical elements.

The foliar application of nutrients should be treated as a complementary or interventional treatment. This method is especially justified during intensive plant growth, when there is the greatest need for nutrients. Bulgari et al. (2015) emphasise the complexity of biostimulant synergistic mechanisms shaping the physiological response of plants and, hence, the difficulties in unambiguously interpreting the basis of these processes.

CONCLUSIONS

1. The combined fertilisation with free amino acids and iron chelate had a positive effect on the visual assessment of the lawns.

2. Fertilisation with amino acids and the combination of amino acids and iron positively influenced the stability of leaf color throughout the growing season resulting in higher evaluations of the functional and aesthetic value of turf grasses.

3. When compared to the control object, the values of vegetation indices LAI, SPAD, and NDVI exhibited a statistically significant increase for the objects where both products and amino acids were applied together.

4. There was no statistically significant effect of the products used (amino acids and iron chelate) on the content of macronutrients and micro-

nutrients, with the exception of iron content. It was observed that iron content significantly altered.

5. The use of Fe chelate significantly increased the content of P and Mg in grasses on the test sites.

Author contributions

Conceptualization: A.R., K.W. and I.R.; methodology: A.R., K.W. and H.B.; software: R.B.; validation: R.B., Ł.J; formal analysis: R.B., Ł.J; investigation: A.R. and I.R.; resources: A.R. and K.W.; data curation: A.R. and K.K.; writing – original draft preparation: A.R., I.R. and H.B.; writing – review and editing: A.R., I.R., Ł.J. and K.K; visualization: A.R., K.K; supervision: A.R. and K.K. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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