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Increase of soybean nutritional quality with non-standard foliar fertilizers

Povećanje nutritivnog kvaliteta soje pomoću nestandardnih folijarnih đubriva

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Abstract

Deficiencies of mineral elements in human nutrition could be surpassed by crop fortification. One of the prevalent measures of fortification is foliar fertilization. The aim of this study was to determine the content and availability of the mineral nutrients Mg, Fe and Zn, together with phytate, as an anti-nutritive factor, and β-carotene as a promoter of mineral nutrient availability in grain of two soybean cultivars (Nena and Laura) treated with different non-standard foliar fertilizers (mainly based on plant extracts). Generally, a negative correlation between Fe and phytate indicated that factors which decrease phytate and increase β-carotene could be primarily responsible for Fe utilization by humans and animals. Zlatno inje (based on manure) had the highest impact on increasing the grain yield and decreasing the ratios between phytate and mineral elements in Nena grain, while for Laura, it was generally Zircon (based on an extract of *Echinacea purpurea* L), increasing also availability of mineral elements.

Keywords: β-carotene, fortification, iron, magnesium, phytate, zinc

Rezime

Nedostatak mineralnih elemenata u ljudskoj ishrani se može prevazići fortifikacijom useva. Najčešće korišćena mera je upotreba folijarnih đubriva. Istraživanje ima za cilj da utvrdi sadržaj i pristupačnost mineralnih nutrienata Mg, Fe i Zn, zajedno sa fitatom kao anti-nutritivnim faktorom i β-karotenom kao promoterom koji utiču na pristupačnost mineralnih nutrienata u semenu dve sorte soje (Nena i Laura) tretiranih različitim folijarnim đubrivima (uglavnom na bazi biljnih ekstrakata). Negativna korelacija između Fe i fitata ukazuje da bi faktori koji smanjuju sadržaj fitata i povećavaju sadržaj β-karotena mogli biti primarno odgovorni za Fe usvajanje kod čoveka i životinja. Zlatno inje (đubrivo na bazi stajnjaka) je pokazalo najveći uticaj na povećanje prinosa i smanjenje odnosa fitata i mineralnih elemenata u zrnu Nene, dok je kod Laure najveći uticaj ispoljio Cirkon (na bazi ekstrakta *Echinacea purpurea* L), povećavajući takođe pristupačnost minerlanih elemenata.

Ključne reči: β-karoten, cink, fitat, fortifikacija, gvožđe, magnezijum

Detaljan rezime

Nedostatak mineralnih elemenata u ljudskoj ishrani se može prevazići povećanjem njihovog sadržaja putem fortifikacije. Folijarna primena nutrienata omogućava njihovu bržu penetraciju kroz list, uz brži odgovor biljke. Biljna hrana može da sadrži anti-nutriente (kao što je fitat) koji onemogućavaju apsorpciju i iskorišćenje mineralnih nutrienata od strane monogastričnih organizama, kao i promotere (kao što je β-karoten) koji povećavaju pristupačnost mineralnih nutrijenata. Cilj ogleda je bio da se odredi sadržaj i potencijalna pristupačnost mineralnih nutrienata Mg, Fe i Zn, zajedno sa fitatom, kao anti-nutritivnim faktorom i β-karotenom, kao promoterom u soji tretiranoj različitim nestandardnim folijarnim đubrivima tokom 2009., 2011. i 2012. godine.

U eksperimentu su korišćene sorte soje: Nena i Laura, kao i folijarna đubriva: Zlatno inje (na bazi stajnjaka), AlgarenB-Zn (na bazi ekstrakta lage *Ecklonia maxima*), Zircon (na bazi ekstrakta biljke *Echinacea purpurea* L.), Eco-Fert (na bazi ekstrakata lekovitih biljaka sa mineralom zeolitom), Agrostemin Zlatni (na bazi samlevenog semena *Agrostema githago* L.), Amalgerol Premium (aminokiselinsko đubrivo), Lithovit Forte (nano-prah $CaCO_3$) i fitohormonski preparat Epin Extra (na bazi 24-epibrassinolida). Nakon žetve određen je sadržaj neorganskog fosfora (P_i), fitinskog fosfora (P_{phy}), ukupnog fosfora (P_{tot}), β-karotena, kao i mineralnih elemenata: Mg, Fe i Zn.

U 2012., kao ekstremnoj godini zabeležene su najviše prosečne temperature uz nizak nivo padavina tokom perioda nalivanja zrna (Tabela 1). Godina, kao i interakcija godine i tretmana su značajno uticali na variranje prinosa kod obe sorte soje, sa nižim vrednostima u 2012. (Tabela 2). U povoljnim godinama najveći prinos zrna je ostvaren u tretmanu sa Zlatnim injem i to u 2009. kod Nene i Zircon-om u 2011. kod Laure. S obzirom da se najveći deo P_{tot} sastoji od P_{phy}, njihove vrednosti su varirale paralelno i značajno pod uticajem godine i interakcije godina x tretman kod Laure, dok je kod Nene samo interakcija pokazala značajan uticaj (Tabela 2). Najmanji P_{phy} je bio u 2009., u tretmanu Agrostemin kod Nene, kao i u 2011., u tretmenu Zircon kod Laure. Sadžaj P_i je značajno varirao pod uticajem interakcije godina x tretman kod obe sorte, kao i pod uticajem godine kod Nene, sa najvećim ostvarenim vrednostima u 2009. (Tabela 3). Najviše vrednosti P_i su dobijene u kontroli za Lauru, a sa Amalgerol Premium đubrivom za Nenu. Sadržaj β-karotena je značajno varirao pod uticajem tretmana i interakcije godina x tretman (Tabela 3), sa najvišim prosečnim vrednostima u tretmanu Lithovit Forte kod Laure i u kontroli kod Nene. Interakcija godina x tretman je takođe pokazala značajan uticaj na variranje sadržaja Mg, Fe i Zn u zrnu obe sorte (Tabela 4). Najveći sadržaj Mg je bio u 2012. pod uticaj Zircon-a kod Laure, kao i pod uticajem Eco-Fert-a kod Nene; najveći sadržaj Fe je bio u zrnu Laure tretirane sa preparatom Epin Extra u 2011., kao i u zrnu Nene u tretmanu Zlatno inje u 2009.; Zn je imao najviše vrednosti u zrnu Laure, u Agrostemin tretmanu (2012.) i u zrnu Nene takođe u tretmanu Zlatno inje (2009.), tretmanu sa najvišim ostvarenim prinosom (Tabela 2). Povoljnija sezona je pokazala pozitivan uticaj na smanjenje odnosa fitata, P_i, β-karotena, Mg, Fe i Zn (Tabela 5), ukazujući na povećanu pristupačnost Pi i mineralnih elemenata. Najveće vrednosti odnosa su uglavnom dobijene u kontroli i Eko-Fert tretmanu. Principal Component Analiza (Tabela 6) ukazuje da su faktori odgovorni za smanjenje sadržaja fitata u zrnu soje odgovorni i za povećanje sadržaja Fe u većem stepenu, dok je pozitivna veza između β-karotena i Mg manje izražena i zavisna je od drugih faktora.

Povoljna sezona je pokazala pozitivan uticaj ne samo na prinos, već i na potencijalnu pristupačnost P_i, Mg, Fe i Zn, sa smanjenjem sadržaja P_{phy} i povećanjem β-karotena u zrnu soje. Pozitivna veza između β-karotena i Mg, kao i negativna korelacija između Fe i fitata ukazuje da faktori koji utiču na smanjenje fitata i u manjem stepenu na povećanje β-karotena bi mogli biti primarno odgovorni za veće Fe i Mg iskorišćenje od strane ljudi i mnogastričnih životinja. Zlatno inje je pokazalo najveći uticaj na povećanje prinosa i smanjenje posmatranih odnosa u zrnu Nene, dok je kod Laure to bio Zircon.

Introduction

Nutritional deficiencies, mainly iron, zinc, vitamin A, etc. account for almost two-thirds of the childhood deaths worldwide (Welch and Graham, 2004). They can be overcome by food supplementation, food fortification or plant breeding (Lönnerdal, 2003; White and Broadley, 2005). Fe and Zn are mineral elements with the greatest significance in vegetarian diets, where meat elimination, together with increased intake of whole grain cereals and legumes (rich in anti-nutrients), significantly decreases their absorption (Hunt, 2003). Fe deficiency, known as anaemia, affects an estimated 30% of the world’s population. Zn is essential for the immune system, activation of many enzymes and growth. Mg concentrations in the human body are insufficient in most cases, what is associated with numerous pathological conditions widely connected with obesity, atherosclerosis, hypertension, osteoporosis, diabetes and cancer (Nielsen, 2010).

About half of all soils are deficient in micronutrients and humans and animals whose diets are mainly based on crops could be deficient (Graham et al., 2007). Incorporation of important mineral elements into soil by fertilizers could be problematic due to their pathway in soil. Fageria et al. (2009) emphasized that foliar application of nutrients is an important management strategy in maximizing crop yields, which could supplement soil fertilization. Foliarly applied nutrients penetrate the leaf rapidly and crop response occurs in a short time. Haq and Mallarino (2000) reported that foliar fertilization of soybean affected nutrient uptake, photosynthesis, grain chemical composition and plant weight. Fageria et al. (2009) indicated that some nutrients when applied foliarly interact positively with other nutrients, increasing their absorption together with grain yield.

However, not all mineral elements in plant foods are bio-available to humans and animals. Plant foods could contain anti-nutrients (e.g., phytate, phenolics, trypsin inhibitors, etc.), which interfere with the absorption or utilization of nutrients. The question is important when enrichment of plant foods with mineral elements is taken into account (including different cropping practices or plant breeding). This implies also to promoters (e.g., ascorbic acid, S-containing amino acids, β-carotene, etc.) that enhance micronutrient bioavailability or decrease anti-nutrient activity (Welch and Graham, 2004).

The aim of the study was to determine the content and potential availability of the mineral nutrients Mg, Fe and Zn, together with phytate as an anti-nutritive factor and β-carotene as promoter in soybean treated with different non-standard foliar fertilizers.

Materials and methods

The trials with two soybean cultivars: Laura (lacks in anti-nutrient Kunitz trypsin inhibitor) and Nena (with a standard grain composition) were set up under rain-fed conditions in Zemun Polje in the vicinity of Belgrade, Serbia, SE Europe (44°52′N, 20°20′E) in following seasons: 2009 (cv. Nena), 2011 (cv. Laura), and 2012 (both cultivars). Soil type was chernozem, with: 0.0% coarse, 53.0% sand, 30.0% silt, 17.0% clay, 3.3% organic matter, pH 7.0 KCl and pH 7.17 H_2O. The texture was silty clay loam containing: 37.45 mg N kg^{-1}, 10.70 mg P kg^{-1}, 107.40 K N kg^{-1}, 327.95 mg Mg kg^{-1} and 0.65 mg Fe kg^{-1} in the 0–30 cm layer. The experiment included application of different foliar fertilizers in the recommended doses at the beginning of flowering (first half of June): Zlatno inje (a liquid fertilizer based on manureand slurry, rich in humic substances, in an amount of 4 L ha$^{–1}$), AlgarenB-Zn (liquid organic fertilizer based on *Ecklonia maxima* algae extract with high contents of B and Zn, in an amount of 0.834 L ha$^{–1}$), Zircon (a liquid fertilizer based on an extract of *Echinacea purpurea* L., a medicinal plant, rich in phenolic acids, in an amount of 0.12 L ha$^{–1}$), Eco-Fert (liquid fertilizer based on extracts of medicinal plants with dissolved mineral zeolite, applied as a 3% aqueous solution), Agrostemin Zlatni (based on milled *Agrostema githago* L. grain - dry plant extract, in an amount of 30 g ha$^{–1}$), Amalgerol Premium (liquid amino acid fertilizer, in amount of 5 L ha$^{–1}$), Lithovit Forte (solid powder of $CaCO_3$ nanoparticles, so called CO_2 fertilizer, applied as an 0.4% aqueous solution), and the phytohormone preparation Epin Extra (based on 24-epibrassinolide, in an amount of 0.136 L ha$^{–1}$) with consumption of 400 L water ha$^{–1}$. The experiments were conducted in the split-plot experimental design in four replications with a 5 × 5 m elementary plot.

After harvesting, the grain yield and contents of different metabolites in soybean grain were determined. The contents of inorganic phosphorus (P_i) and phytic phosphorus (P_{phy}) were determined colorimetrically after extraction with 5% trichloro-acetic acid, using the method of Dragicevic et al. (2011): P_{phy} was determined with Wade reagent and P_i with vanado-molybdate reagent. The β-carotene content was also determined colorimetrically, after extraction with saturated n-butanol (AACC, 1995). The content of total phosphorus (P_{tot}) was analysed by the vanado-molybdate method after wet digestion with $HClO_4$+HNO_3, according to Pollman (1991). From the same digested samples, the following elements were determined by Inductively Coupled Plasma - Optical Emission Spectrometry: Mg, Fe and Zn.

The experimental data were statistically processed by analysis of the variance (ANOVA) and analyzed by the LSD-test (5%), as well as by the coefficient of variation and Principal Component Analysis (PCA) in SPSS 15.0 for Windows Evaluation version.

Table 1. Meteorological conditions during the growth season of 2009, 2011 and 2012

Tabela 1. Meteorološki uslovi tokom vegetacione sezone 2009., 2011. i 2012.

Year	Months						$\bar{x}$ - Σ
	IV	V	VI	VII	VIII	IX	
			Temperature (°C)				
2009	15.1	18.9	20.4	23.2	23.1	20.1	20.1
2011	13.4	16.8	21.5	23.3	23.9	21.6	20.1
2012	14.5	17.9	24.6	27.1	26.2	22.1	22.1
			Precipitation (mm)				
2009	6.8	41.3	86.8	55.16	53.9	11.0	254.9
2011	14.9	89.6	26.2	44.0	66.0	32.6	273.3
2012	66.7	127.5	13.9	39.4	4.0	31.4	282.9

Meteorological conditions indicated that 2012 was the year with the highest average temperature (Table 1). August 2012 was characterised with only 4 mm of precipitation, which together with average temperatures of 27.1 and 26.2 during July and August, respectively, signified this period as stressful (for SE Europe agro-ecological conditions). Other than this, 2011 was characterised with relative uniform precipitation schedule, while 2009 was dry during the sprouting period and beginning of growth (April and May, with 6.8 and 41.3 mm, respectively).

Results and discussion

According to results in Table 2, the year and the interaction between year and treatment significantly affected the grain yield of both cultivars, with significantly lower values obtained in 2012, owing to the unfavourable meteorological conditions. In the favourable years, the highest grain yield was achieved with the Zlatno inje treatment in 2009 for Nena and with Zircon treatment in 2011 for Laura, which are about 11–12% higher, compared to the control in the same year. According to Haq and Mallarino (2000) and Fageria et al. (2009) foliar application of nutrients is an important strategy for maximizing crop yields, with inconsistent grain yield increases in soybean, which was noticeable in the variations of grain yield between the applied treatments in range of 21–25% on average for Laura and Nena.

Table 2. The effects of applied non-standard foliar fertilizers on grain yield and the contents of total phosphorus (P_{tot}) and phytic phosphorus (P_{phy}) in the grain of two soybean cultivars

Tabela 2. Efekat nestandardnih folijarnih đubriva na prinos zrna i sadržaj ukupnog fosfora (P_{tot}) i fitinskog fosfora (P_{phy}) u zrnu dve sorte soje

Treatm.	Grain yield (t ha^{-1})			P_{tot} (g kg^{-1})			P_{phy} (g kg^{-1})		
	2011	2012	$\bar{x}$	2011	2012	$\bar{x}$	2011	2012	$\bar{x}$
					LAURA				
Control	4.41	1.80	3.10	17.35	20.59	18.97	14.25	17.03	15.64
Agrostemin	4.39	1.82	3.10	17.20	19.70	18.45	13.78	15.80	14.79
Algaren B-Zn	4.03	2.49	3.26	17.21	19.10	18.16	14.06	15.75	14.91
Lithovit Forte	4.08	2.40	3.24	17.63	18.30	17.96	13.93	15.30	14.62
Epin Extra	4.50	1.94	3.22	16.60	18.96	17.78	13.62	15.45	14.53
Zircon	4.99	2.81	3.90	16.56	17.93	17.25	13.27	15.13	14.20
$\bar{x}$	4.40	2.21	3.30	17.09	19.10	18.10	13.82	15.75	14.78
LSD 0.05	Year	Treat.	YxT	Year	Treat.	YxT	Year	Treat.	YxT
	0.78	1.43	0.83	0.72	1.26	0.18	0.53	1.15	0.14
					NENA				
Control	4.06	2.24	3.15	19.73	18.65	19.19	16.79	15.64	16.22
Agrostemin	3.76	2.16	2.96	19.96	19.46	19.71	15.01	15.86	15.43
Amalg. premium	4.11	2.96	3.54	19.38	19.89	19.64	15.32	16.65	15.99
Eko-Fert	3.11	2.81	2.96	19.29	19.89	19.59	15.78	17.07	16.43
Zlatno inje	4.58	3.34	3.96	19.68	19.32	19.50	16.78	15.16	15.97
$\bar{x}$	3.93	2.70	3.31	19.61	19.44	19.52	15.94	16.08	16.01
LSD 0.05	Year	Treat.	YxT	Year	Treat.	YxT	Year	Treat.	YxT
	0.82	1.01	0.79	0.40	0.39	0.10	1.01	1.04	0.9

Considering that the majority of the P_{tot} in the grain consisted of P_{phy}, their values varied in parallel and significantly under the influence of year and the interaction of year × treatment for Laura, while for Nena, only the interaction year × treatment had a significant impact (Table 2). In Laura grain, the highest P_{tot} and P_{phy} were registered in the control of 2012, while in Nena grain, Eco-Fert in 2012 exhibited a positive impact on the P_{phy} increase. Cakmak (2008) emphasised that an increased P uptake is due to the high phloem P mobility and most of the accumulated P in the grain is converted into phytic acid. Other than that, the lowest P_{phy} was observable in the meteorologically favourable year, in 2009, and with the Agrostemin treatment for Nena, as well as 2011 and with the Zircon treatment for Laura, which are about 12% and 22% lower, compared to the highest P_{phy} value, respectively.

Table 3. The effects of applied non-standard foliar fertilizers on inorganic phosphorus (P_i) and β-carotene content in the grain of two soybean cultivars

Tabela 3. Efekat nestandardnih folijarnih đubriva na sadržaj neorganskog fosfora (P_i) i β-karotena u zrnu dve sorte soje

Treatment	P_i (g kg^{-1})			β-carotene (mg kg^{-1})		
	2011	2012	$\bar{x}$	2011	2012	$\bar{x}$
LAURA						
Control	0.524	0.457	0.491	12.79	10.99	11.89
Agrostemin	0.497	0.327	0.412	12.37	13.32	12.84
Algaren B-Zn	0.500	0.475	0.488	12.93	11.99	12.46
Lithovit Forte	0.512	0.481	0.496	13.41	12.33	12.87
Epin Extra	0.462	0.474	0.468	11.02	11.44	11.23
Zircon	0.446	0.463	0.454	12.19	10.33	11.26
$\bar{x}$	0.490	0.446	0.468	12.45	11.73	12.09
LSD 0.05	Year	Treat.	YxT	Year	Treat.	YxT
	0.045	0.044	0.006	0.92	0.76	0.22
NENA						
Control	0.582	0.495	0.539	14.16	14.69	14.42
Agrostemin	0.761	0.406	0.583	13.75	10.94	12.35
Amalg. premium	0.789	0.312	0.550	12.10	13.27	12.69
Eko-Fert	0.674	0.457	0.565	13.23	12.42	12.82
Zlatno inje	0.827	0.465	0.646	14.19	14.63	14.41
$\bar{x}$	0.73	0.43	0.58	13.49	12.81	13.34
LSD 0.05	Year	Treat.	YxT	Year	Treat.	YxT
	0.083	0.190	0.021	1.20	0.84	0.15

The P_i content was significantly affected by the year × treatment interaction for both cultivars, and by the year for Nena, having higher values in 2009 (Table 3). Increased P_i and decreased P_{phy} content in favourable years should also be taken into consideration, parallel with a further P_{phy} decrease by breeding (Dragicevic et al., 2013; Lönnerdal, 2003). For both cultivars, in the favourable year, higher P_i values were achieved in the control in the Laura grain and with Amalgerol Premium in the

Nena grain (26% higher, compared to the control). The content of β-carotene was mainly affected by the treatment and the interaction year × treatment (Table 3), having the highest average value with Lithovit Forte treatment for Laura (8% higher, in relation to the control) and in the control for Nena. The interaction also stressed Lithovit Forte in 2011 and the control in 2012 for Laura and Nena, respectively, with the highest β-carotene content in the grain.

Table 4. The effects of the applied non-standard foliar fertilizers on the content of Mg, Fe and Zn in the grain of two soybean cultivars

Tabela 4. Efekat nestandardnih folijarnih đubriva na sadržaj Mg, Fe i Zn u zrnu dve sorte soje

Treatment	Mg (mg kg^{-1})			Fe (mg kg^{-1})			Zn (mg kg^{-1})		
	2011	2012	$\bar{x}$	2011	2012	$\bar{x}$	2011	2012	$\bar{x}$
					LAURA				
Control	2037	1969	2003	73.75	53.22	63.48	40.63	36.75	38.69
Agrostemin	2075	2172	2124	75.00	58.00	66.50	50.63	59.72	55.17
Algaren B-Zn	2110	2216	2163	78.75	60.09	69.42	44.69	56.59	50.64
Lithovit Forte	2099	2138	2118	72.50	55.16	63.83	42.81	54.81	48.81
Epin Extra	2139	2181	2160	83.75	57.13	70.44	49.69	45.03	47.36
Zircon	2195	2235	2215	80.63	66.13	73.38	45.00	50.81	47.91
$\bar{x}$	2109	2152	2130	77.40	58.29	67.84	45.57	50.62	48.10
LSD 0.05	Year	Treat.	YxT	Year	Treat.	YxT	Year	Treat.	YxT
	75.9	44.5	21.2	4.36	11.31	1.39	7.60	6.83	5.78
					NENA				
Control	2137	2037	2087	56.28	56.41	56.34	31.22	37.44	34.33
Agrostemin	2184	2106	2145	65.88	59.97	62.92	37.63	35.63	36.63
Amalg. prem.	2150	2050	2100	62.38	57.69	60.03	36.69	32.50	34.59
Eko-Fert	2150	2222	2186	56.97	57.34	57.16	35.47	35.13	35.30
Zlatno inje	2203	2122	2162	66.13	64.34	65.23	40.50	33.16	36.83
$\bar{x}$	2165	2107	2136	61.53	59.15	60.34	36.30	34.77	35.53
LSD 0.05	Year	Treat.	YxT	Year	Treat.	YxT	Year	Treat.	YxT
	57.7	57.0	33.1	3.91	2.25	1.36	4.01	4.32	4.08

The type of treatment was also a source of Mg and Zn variation in Laura grain, while the year had a significant impact on Fe variation (Table 4). For Nena, the type of treatment induced significant variations in the Fe content. The interaction year × treatment also showed significant impact on the Mg, Fe and Zn variations in the grain of both cultivars. The highest Mg content was in 2012 with Zircon treatment in Laura grain and in Eco-Fert treatment in Nena grain; the highest Fe content was observed in the grain of the Laura cultivar treated with Epin Extra in 2011, as well as in Nena grain after application of Zlatno inje treatment in 2009; Zn had the highest value in Laura grain with Agrostemin treatment (2012) and in Nena grain with Zlatno inje treatment also (2009), the same treatment with the highest grain yield (Table 2), indicating the positive relationship between increases of the Zn concentration in grain and grain yield increases (Inocêncio et al., 2012).

Parallel with P_{phy} decreases (Table 2), some foliar fertilizers mainly showed positive impacts on Mg and Fe increases (Table 4), i.e., in Zircon treatment for Laura and Zlatno inje treatment for Nena, in both experimental years. This could be positively connected with the improved Mg and Fe status (Lönnerdal, 2003). Luo and Xie (2012) found that food rich in β-carotene could significantly enhance Fe and Zn bioavailability from grain. In the present study, Agrostemin mostly contributed to parallel increases of β-carotene and Zn in Laura grain, while Zlatno inje had the same impact in Nena grain.

Variations in the ratios between phytate, β-carotene and the mineral nutrients could indicate possible bio-availability of the mineral nutrients (Walter Lopez et al., 2002; Dragičević et al., 2013), with increased tendency of availability with decreasing ratio. The favourable year showed a positive impact on decreasing the ratios between phytate and P_i, β-carotene, Mg, Fe and Zn (Table 5), indicating increased availability of P_i and the mineral elements, which was supported by the results of Lönnerdal (2003), who ascertained that any reduction in phytic acid content in food is likely to result in improved statuses of Mg, Fe and Zn. Cakmak (2008) also underlined that the phytate-Zn molar ratio is a widely used criterion for estimating the bioavailability of Zn in diets. The highest values obtained for the ratios were mainly realised in Laura grain in the control, while in Nena grain, it was mainly realised in the control and Eco-Fert treatment. Zlatno inje and Amalgerol Premium decreased mainly the P_{phy}/P_i and Phy/β-carotene ratios in Nena grain, while in Laura grain, Lithovit Forte was the most effective in decreasing the ratios. Moreover, Eco-Fert decreased the Phy/Mg, Phy/Fe and Phy/Zn ratios, mainly in 2009. There was no consistency in the revealed effect of the applied fertilizers on the Phy/Mg, Phy/Fe and Phy/Zn ratio, with the lowest value of the Phy/Mg ratio obtained with the Zircon, the Phy/Fe ratio with the Epin Extra and the Phy/Zn ratio with the Agrostemin treatment.

Table 5. Molar ratios between phytate (Phy), inorganic P (P_i), β-carotene, Mg, Fe and Zn in the grain of two soybean cultivars

Tabela 5. Molarni odnosi između fitata (Phy), neorganskog P (P_i), β-karotena, Mg, Fe i Zn u zrnu dve sorte soje

Treatment	P_{phy}/P_i		Phy/β-carotene		Phy/Mg		Phy/Fe		Phy/Zn	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
					LAURA					
Control	27.2	37.3	2124	2956	2.10	2.60	58.1	96.2	123.4	163.1
Agrostemin	27.7	48.3	2124	2262	2.00	2.19	55.2	81.9	95.8	93.1
Algaren B-Zn	28.1	33.2	2074	2504	2.00	2.14	53.7	78.8	110.7	97.9
Lithovit Forte	27.2	31.8	1981	2367	1.99	2.15	57.7	83.4	114.5	98.2
Epin Extra	29.5	32.6	2356	2575	1.91	2.13	48.9	81.3	96.4	120.7
Zircon	29.7	32.7	2075	2794	1.82	2.03	49.4	68.8	103.7	104.8
$\bar{x}$*	28.2±3.9	36.0±17.6	2122±5.9	2576±10.1	1.97±4.9	2.21±9.0	53.8±7.4	81.7±10.8	107.4±10.1	113.0±23.2
					NENA					
Control	28.8	31.6	2260	2031	2.36	2.31	89.7	83.3	189.2	147.0
Agrostemin	23.4	37.4	2275	2621	2.21	2.31	83.3	89.5	156.6	171.0
Amalg. prem.	20.3	32.6	2254	1976	2.29	2.15	76.2	70.8	145.7	160.8
Eko-Fert	19.7	39.1	2080	2764	2.06	2.26	68.5	79.5	140.3	156.6
Zlatno inje	19.4	53.4	2415	2391	2.14	2.44	73.8	86.7	146.9	180.2
$\bar{x}$*	22.3±17.8	38.8±22.5	2257±5.3	2357±14.8	2.21±5.3	2.29±4.6	78.3±10.6	82.0±8.9	155.8±12.6	163.1±7.9

* $\bar{x}$ ± Coefficient of variation (%)

In parallel, PCA showed that P_{phy} and Fe mostly contributed to PC1, while Mg mostly contributed to PC2, whereas β-carotene and Mg contributed mostly to PC3 (Table 6). This could indicate that factors which are responsible for a decrease of the phytate content in soybean grain could also increase the Fe content to a high degree (explained variance of 2.185), while β-carotene and Mg were positively related, but to a lesser extent (explained variance of 0.884), and were influenced by other factors. Moreover, studies with maize and beans did not support a lower Fe availability from grains with a slightly higher phytate content (Beiseigel et al., 2007), while significance was given to factors that improve Fe availability, such as ascorbic acid, as well as β-carotene, which was included in the present study.

Table 6. Results of PCA for P_{phy}, β-carotene and mineral elements content in the grain of two soybean cultivars (synthetic variables: PCA1 - principal component axis 1, PCA2 - principal component axis 2 and PCA3 - principal component axis 3)

Tabela 6. Rezultati PCA za sadržaj P_{phy}, β-karotena i mineralnih elemenata u zrnu dve sorte soje (sintetičke varijable: PCA1 - osa 1 analize glavnih komponenata, PCA2 - osa 2 analize glavnih komponenata i PCA3 - osa 3 analize glavnih komponenata)

	PCA1	PCA2	PCA3
P_{phy}	0.597	-0.353	-0.042
β-carotene	0.285	0.437	0.739
Mg	-0.186	-0.594	0.661
Fe	-0.565	0.389	0.124
Zn	-0.457	-0.426	-0.017
Explained variance	2.185	1.272	0.884
Proportion of total variance (%)	43.7	25.4	17.7

Based on the obtained results, it could be concluded that the favourable season had a positive impact not only on the yield potential, but also on the potential availability of P_i, Mg, Fe and Zn, while decreasing the P_{phy} and increasing the β-carotene content in grain. β-carotene and Mg were positively related and the mainly negative correlation between Fe and phytate indicated that factors which reduce the phytate content and, to some extent, increase the β-carotene content, could be primarily responsible for Fe and Mg utilization by humans and animals. There was no consistency in the revealed effect of the applied fertilizers on the Phy/Mg, Phy/Fe and Phy/Zn ratios in the grain of either cultivar. However, Zlatno inje had the highest impact on increasing the grain yield and decreasing the observed ratios in Nena grain, while for Laura, it was generally Zircon.

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