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Alterations in mineral nutrients in soybean grain induced by organo-mineral foliar fertilizers

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Abstract

Background: Chemical composition of soybean grain may be modified by application of foliar fertilizers. The aim of this study was to test the effect of different organo-mineral foliar fertilizers: Zlatno inje, Bioplant Flora, Algaren BZn, Zircon, as well as plant growth regulator Epin Extra, on potential availability of mineral elements (Mg, Fe, Mn and Zn) from grain of three commercial soybean varieties: ZP-015, Nena and Laura (variety lacking in Kunitz trypsin inhibitor). In addition, phytate (Phy) and β-carotene contents were determined.

Results: ZP-015 achieved the highest P, Mg, Fe, Mn and β-carotene contents. Laura had the highest Phy level, which might reflect the diminished availability of nutrients from grain. Compared to control, most of the applied fertilizers increased β-carotene and decreased Mn content in all three soybean varieties. Increase in β-carotene content was followed by increase in Fe content, mainly in grains with larger weight, as a part of improved yielding potential.

Conclusions: Positive effect of Zircon application was evident on increased grain weight, and β-carotene and Fe content. These parameters together with the lowest values found for Phy/β-carotene and Phy/Mg ratios may explain the enhanced Mg and Fe bioavailability. On the other hand, positive effects of Epin Extra were mostly reflected by a decrease of Phy and an increase in Fe and Mn, thus becoming more bio-available. Accordingly, the organo-mineral foliar fertilizers based mainly on phenolic acids (Zircon) and bioregulator (Epin Extra) are to be recommended for soybean fortification.

Keywords: Organo-mineral foliar fertilizer; Grain composition; Mineral elements; Phytic phosphorus; *Glycine max* (L.) Merr

Background

Nutrition is crucial factor in reduction of hunger, malnutrition and obesity [1]. Human body requires more than 22 mineral elements, which can be provided by adequate diet. On the other hand, nutritional deficiencies (e.g. in iron, zinc, vitamin A) account for almost two-thirds of the childhood deaths worldwide [2]. These deficiencies can be surpassed by increase of mineral nutrients in food through supplementation, food fortification or plant breeding [3,4].

Iron and zinc are considered to be the most important mineral elements in vegetarian diets. Elimination of meat from diet, along with increased intake of whole grain cereals and legumes rich in anti-nutrients, like phytate, significantly decrease Fe and Zn absorption [5]. The most prevalent among mineral elements deficiencies is Fe deficiency (anemia), affecting approximately 30% of the world's population. Zn is essential element, involved in the immune system, activation of many enzymes and the growth. Zn deficiency has been detected in cases of inadequate dietary supply, abnormal blood losses or high physiological requirements for growth, as well as during puberty, pregnancy and lactation [4,5]. As a part of the antioxidant system of defense in mitochondria, manganese is also essential element for humans and is involved in metabolism, bone development and wound healing. It has been shown that Mg has protective role against various diseases. However, numerous studies indicated that Mg concentration in human body is usually insufficient [6]. According to Nielsen [7], low level of Mg has been associated with pathological conditions characterized as a chronic inflammatory stress, being widely associated with obesity, atherosclerosis, hypertension, osteoporosis, diabetes mellitus, and cancer.

According to present knowledge, it is necessary to increase content of mineral nutrients in edible parts of plants. Accumulation of mineral elements in seeds and grains is controlled by a number of processes including

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root-cell uptake, root-shoot transfer, and the ability to deliver these nutrients to developing seeds and grains [8]. Designing of cultivation systems, in order to improve nutrition and health, should become an integral part of goals in modern agriculture. It is mainly concerned to cultivation on poor soils, where micronutrient element enhancement can contribute to increased crop yield. According to Graham *et al.* [9], probably half of all soils are deficient in micronutrients and even though plant production is not limited, humans and animals whose diets are mainly based on crops can be potentially deficient in essential micronutrients. Incorporation of important mineral elements into soil by fertilizers could be problematic due to their pathway in soil. For instance, Fe from fertilizers could be quickly oxidized and became insoluble in soil, so Fe deficiency is mainly a consequence of Fe deficient soils [9]. Welch [8] reported significant impact of fertilizers containing N, P, K, S and Zn on accumulation of nutrients in edible plant products, including grains. Other micronutrient fertilizers were shown to have very small effect on the amount of micronutrients accumulated in edible seeds and grains when applied to soils.

Increased content of mineral elements in crops presents only the first step in making them improved sources of nutrients for humans [10], since not all mineral elements in plant foods are bio-available to humans and animals. Plant food can contain anti-nutrients, which interfere with the absorption of mineral nutrients in humans and animals. The question of bio-availability must be taken into consideration when enrichment of plant food with mineral elements was employed. This also takes into account enhancing substances - promoters (e.g. ascorbic acid, S-containing amino acids, etc.) that promote micronutrient bioavailability and/or suppress anti-nutrient substances (e.g. phytate, polyphenolics, etc.) that inhibit micronutrient bioavailability [2,11]. Thus, it is essential to decrease content of various anti-nutrients in foods and to increase content of promoters [9].

Phytic acid - Phy (myo-inositol 1,2,3,4,5,6-hexakisphosphate) is the major phosphorus storage compound in grains (accounting for up to 80% of total P) and it can acts as anti-nutritional factor that chelate essential elements including Ca, Zn and Fe [12]. As content of phytic acid in diet increases, the intestinal absorption of Zn, Fe and other mineral nutrients decreases [12], while the reduction in phytic acid content in food is likely to result in improved Fe, Zn and Mn content [3,13]. β-carotene is considered to be a promoter due to positive effect on mineral nutrients absorption. Lönnerdal [3] stated that β-carotene can enhance Fe absorption in humans. Luo and Xie [14] found that addition of food rich in β-carotene or pure β-carotene, can significantly enhance Fe and Zn bioavailability from the grains. Moreover, Noh and Koo [15] reported that low β-carotene absorption is associated with low Zn intake or slight Zn deficiency. Different cultivation practices, including macronutrient treatments (N, P and Mg), can result in increased concentration of β-carotene (by 42%) and micronutrients in carrots [8].

Soybean is important dietary source of proteins, lipids, minerals, vitamins, fiber and bioactive compounds. However, commonly high levels of phytate in soybean grain could negatively affect its nutritive value. Variability of mineral elements in soybean grain is significant and it also depends on applied cultivation systems [16,17]. Since Zn bioavailability from some soya products is low, application of an adequate cultivation system becomes important. However, compared to other plant foods with lower phytate contents, the Fe availability from soya flour and soya isolates is higher.

The aim of this experiment was to investigate the effect of applied foliar fertilizers on mineral nutrients content (i.e. Mg, Fe, Mn and Zn), along with contents of phytate as anti-nutritive factor and β-carotene as promoter, in chosen soybean varieties differing in chemical composition of grain.

Experimental

Plant material

Two commercial soybean varieties with standard grain composition - ZP-015 and Nena, and the variety lacking in Kunitz trypsin inhibitor - Laura, were the objectives of the present study.

Soil

The field trial was carried out in Zemun Polje (44°52'N 20° 20'E), vicinity of Belgrade, Serbia (in rain-fed conditions). Soil was a slightly calcareous chernozem with 0.0 % coarse, 53.0 % sand, 30.0 % silt, 17.0 % clay, 3.3 % organic matter, 7.0 pH KCl and 7.17 pH H_2O. The texture was silty clay loam, containing: 37.45 mg kg^{-1} N, 10.70 37.45 mg kg^{-1} P, 107.40 37.45 mg kg^{-1} K, 327.95 37.45 mg kg^{-1} Mg, 0.65 37.45 mg kg^{-1} Fe and < 0.02 37.45 mg kg^{-1} Zn in 0–30 cm layer, before fertilizer application. A split-plot experimental design in four replications was used in the experiment. Size of elementary plot was 5 m x 5 m.

Foliar fertilizers

Experimental trial included application of different foliar fertilizers in recommended doses, at the beginning of flowering (first half of June): 1. Zlatno inje (liquid extract of cow's manure, with 0.8% of organic matter, 0.004% N and 0.0004% P), in amount of 4 L ha^{-1}; 2. Bioplant Flora (organic fertilizer with 8% humic acids, isolated from vermicompost, with 1.0% N, 1.5% P, 48.35 mg L^{-1} Mg, 2.41 mg L^{-1} B, 13.14 mg L^{-1} Cu, 212.8 mg L^{-1} Zn, 1.64 mg L^{-1} Co, 462 mg L^{-1} Mn, 775.6 mg L^{-1} Mo and 500 mg L^{-1} Fe), in the amount of 1 L ha^{-1}; 3. AlgarenBZn (organic fertilizer based on *Ecklonia maxima* algae extract with 2% of B and 3% of Zn), in an amount of

0.834 L ha^{-1}; 4. Zircon (extract of medicinal plant *Echinacea purpurea* L., that contains a mixture of 0.1 g L^{-1} of phenolic acids: 3,4-dihydroxycinnamic (caffeic) acid (IUPAC: 3-(3, 4-dihydroxyphenyl)-2-propenoic acid; CAS No 331-95-5), chlorogenic acid (IUPAC: (1*S*,3*R*,4*R*,5*R*)-3-{[(2*Z*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy}-1,4,5-trihydroxycyclohexanecarboxylic acid; CAS No 327-97-9), cichoric acid (IUPAC: (2*R*,3*R*)-2,3-bis{[(*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy}butanedioic acid; CAS No 327-97-9), as active ingredients identical to *Echinacea purpurea* L. plant extract), in the amount of 0.12 L ha^{-1}; 5. plant growth regulator Epin Extra (based on 0.025 g L^{-1} of 24-epibrassinolide (IUPAC: (22R 23R 24S)-2α, 3α, 22, 23 tetra hydroxy-24-metyl 5α-holestan-6-on; CAS No 72962-43-7), in the amount of 0.136 L ha^{-1}. All these organo-mineral fertilizers were applied with a dose of 400 L ha^{-1} of water.

Methods

Chemical analyses

After harvesting, 1,000 grain weight was measured and contents of different metabolites in soybean grain were determined. Contents of inorganic phosphorus (P_i) and phytic phosphorus (P_{phy}) were determined colorimetrically after extraction with 5% trichloroacetic acid, by method of Dragicevic *et al.* [18]: P_{phy} was determined with Wade reagent and P_i with vanado-molybdate reagent. β-carotene content was also determined colorimetrically, after extraction with saturated butanol [19]. Content of total phosphorus (P_{tot}) was analysed with vanado-molybdate colorimetric method after wet digestion with $HClO_4$ + HNO_3, by method of Pollman [20]. The same digested samples were used for determination of mineral elements (i.e. Fe, Mn, Zn, and Mg) by Inductively Coupled Plasma - Optical Emission Spectrometry.

Statistical analysis

All analyses were performed in four replicates (n = 4) and the results were presented as mean ± standard deviation (SD). The differences among soybean varieties and applied treatments, based on mean values of observed parameters, were evaluated by using Principle Component Analysis (PCA). Statistical analysis was performed by SPSS 15.0 for Windows Evaluation version. Correlation analyses were performed using Pearson's correlation coefficient.

Results and Discussion

Grain weight and chemical composition of the grain

Results presented in Table 1 indicated that the greatest average 1,000 grain weight was achieved by the Laura

Table 1 The effect of different foliar fertilizers on chemical composition of grain in three soybean varieties

	Treatment	1,000 grain weight (g)	P_{tot}** (g kg^{-1})	P_i (g kg^{-1})	P_{phy} (g kg^{-1})	β-carotene (mg kg^{-1})
ZP-015	Control	178.1 ± 12.1*	16.12 ± 0.08	0.30 ± 0.01	12.88 ± 0.23	13.53 ± 0.04
	Zlatno inje	198.2 ± 6.7	16.87 ± 0.00	0.38 ± 0.03	12.71 ± 0.02	11.95 ± 0.19
	Epin Extra	180.9 ± 9.4	16.53 ± 0.03	0.30 ± 0.03	12.14 ± 0.16	13.92 ± 0.17
	Zircon	174.2 ± 6.9	16.25 ± 0.03	0.34 ± 0.03	12.06 ± 0.05	18.73 ± 0.09
	Bioplant Flora	182.7 ± 10.5	16.96 ± 0.11	0.38 ± 0.01	12.59 ± 0.35	15.96 ± 0.06
	AlgarenBZn	176.4 ± 11.9	17.12 ± 0.03	0.46 ± 0.00	12.61 ± 0.20	14.87 ± 0.09
	Average	181.7 ± 9.6	16.64 ± 0.04	0.36 ± 0.02	12.50 ± 0.17	14.83 ± 0.11
Nena	Control	171.9 ± 13.5	13.69 ± 0.08	0.49 ± 0.02	13.49 ± 0.03	14.84 ± 0.11
	Zlatno inje	170.4 ± 6.0	14.27 ± 0.11	0.47 ± 0.00	12.50 ± 0.36	10.94 ± 0.17
	Epin Extra	172.9 ± 8.7	15.42 ± 0.11	0.47 ± 0.00	12.33 ± 0.40	14.08 ± 0.11
	Zircon	191.0 ± 7.0	14.40 ± 0.00	0.29 ± 0.01	12.70 ± 0.16	12.40 ± 0.13
	Bioplant Flora	172.6 ± 8.8	14.38 ± 0.08	0.31 ± 0.02	12.43 ± 0.14	15.78 ± 0.19
	AlgarenBZn	171.1 ± 8.2	14.97 ± 0.03	0.32 ± 0.02	12.95 ± 0.25	12.61 ± 0.06
	Average	175.0 ± 8.7	14.52 ± 0.07	0.39 ± 0.01	12.73 ± 0.22	13.44 ± 0.13
Laura	Control	207.0 ± 11.4	14.68 ± 0.13	0.46 ± 0.00	12.46 ± 0.00	10.99 ± 0.11
	Zlatno inje	194.9 ± 6.7	14.29 ± 0.08	0.41 ± 0.03	12.43 ± 0.20	12.22 ± 0.11
	Epin Extra	197.5 ± 8.7	14.79 ± 0.00	0.47 ± 0.00	12.18 ± 0.00	11.44 ± 0.02
	Zircon	206.4 ± 5.5	14.13 ± 0.24	0.46 ± 0.00	11.93 ± 0.01	12.93 ± 0.06
	Bioplant Flora	208.1 ± 10.2	13.67 ± 0.19	0.49 ± 0.00	12.36 ± 0.01	10.33 ± 0.09
	AlgarenBZn	207.9 ± 8.8	14.08 ± 0.03	0.48 ± 0.00	12.42 ± 0.06	11.99 ± 0.21
	Average	203.6 ± 8.5	14.27 ± 0.11	0.46 ± 0.01	12.29 ± 0.05	11.65 ± 0.10

*The results are represented as mean ± SD (standard deviation) in four replicates.

**P_{tot}, total P; P_i, inorganic P; P_{phy}, phytic P.

variety (25.25 g larger than ZP-015 and Nena). Sudarić *et al.* [21] indicated that 1,000 grain weight is a very important yielding parameter, so that its increase should be considered as the main target for any applied cultivation measure. Compared to control, an average 1,000 grain weight increase of 4.49 g was achieved in Zircon treatment, for all three genotypes. Concerning the individual effect of each applied fertilizer, Zlatno inje increased the 1,000 grain weight mainly in ZP-015, while Epin Extra did so in Nena, and Bioplant Flora in the Laura variety.

The largest average P_{tot} was recorded in ZP-015 (2.02 g kg^{-1} greater than Nena and Laura), as presented in Table 1. A significant effect of fertilizers on increased P_{tot} content was obtained by Algaren BZn, mainly exhibited in grains of ZP-015 and Nena, while the P_{tot} content increase in Laura grain was achieved only by applying Epin Extra. It is known that the majority of P pool in soybean grain is present as P_{phy}, while the minor part is due to inorganic P_i. It is interesting to underline that in grain of ZP-015, only 75% of average P_{tot} consisted of P_{phy}, while it was 86-88% in other two varieties. Nevertheless, such decreased P_{phy} in ZP-015 did not determine an increase of P_i, that is the main source of available P from grains. Hence this genotype may be considered for further P_{phy} decrease by breeding [3,22]. The largest average content of P_{phy} was found in Nena grain, with slight variations of P_{phy} and P_i content in response to the applied foliar fertilizers. Bioplant Flora and Algaren BZn induced slight increase in P_i content in grain of ZP-015 and Laura, while Epin Extra and Zircon decreased P_{phy} in grains of all three varieties. It is also noticed that the largest average P_{phy} was found in control. In relation to P_{phy}, β-carotene variation was to a larger extent, with the largest average values observed in ZP-015 grain. Among the applied fertilizers, the greatest increase in β-carotene content was achieved by the Zircon application on ZP-015 and Laura, and by Bioplant Flora on Nena grain.

Investigated soybean varieties differed to a larger extent in mineral composition of their grain. The largest average Mg, Fe and Mn content was observed in ZP-015 grains, while the relatively greatest average Zn content was found in the Laura grains (Table 2). In parallel with P_{phy} decrease (Table 1), foliar fertilizers mainly exhibited a positive impact on the increase of mineral nutrients content. This could be positively related to a larger Fe, Zn and Mn status [3,13]. Compared to control and for all three genotypes, average increase for Mg and Zn contents (by 3% and 20%, respectively) was found under Bioplant Flora, for Fe content (7%) under Zircon, and

Table 2 The effect of different foliar fertilizers on mineral element contents in soybeans grain

	Treatment	Mg (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})
ZP-015	Control	2284.4 ± 48.6*	65.66 ± 0.49	29.66 ± 1.37	34.41 ± 2.78
	Zlatno inje	2331.3 ± 0.0	70.13 ± 0.18	29.97 ± 1.46	45.41 ± 3.23
	Epin Extra	2459.4 ± 4.4	71.47 ± 0.84	31.53 ± 0.31	36.00 ± 4.15
	Zircon	2215.6 ± 39.8	78.41 ± 0.35	25.78 ± 0.57	48.13 ± 2.08
	Bioplant Flora	2371.9 ± 30.9	68.69 ± 1.99	26.75 ± 0.35	44.91 ± 1.15
	AlgarenBZn	2356.3 ± 35.4	67.91 ± 0.66	28.56 ± 1.33	38.13 ± 2.12
	Average	2336.5 ± 26.5	70.38 ± 0.75	28.71 ± 0.90	41.16 ± 2.59
Nena	Control	2221.9 ± 13.3	57.34 ± 0.40	26.00 ± 0.00	37.44 ± 2.92
	Zlatno inje	2106.3 ± 35.4	60.75 ± 2.25	23.56 ± 1.02	35.63 ± 0.09
	Epin Extra	2162.5 ± 17.7	64.34 ± 0.80	26.28 ± 0.53	32.09 ± 0.13
	Zircon	2321.9 ± 39.8	62.31 ± 0.62	23.78 ± 0.49	35.09 ± 2.34
	Bioplant Flora	2320.9 ± 39.8	63.16 ± 0.75	22.97 ± 1.64	40.38 ± 2.65
	AlgarenBZn	2181.3 ± 0.0	58.81 ± 0.62	21.94 ± 0.09	30.56 ± 3.36
	Average	2219.1 ± 24.3	61.12 ± 0.91	24.09 ± 0.63	35.20 ± 1.92
Laura	Control	2172.5 ± 25.6	60.09 ± 1.37	26.59 ± 1.02	42.84 ± 2.52
	Zlatno inje	2215.9 ± 13.3	66.13 ± 0.22	27.31 ± 0.75	59.72 ± 8.62
	Epin Extra	2234.7 ± 45.1	59.34 ± 1.06	25.81 ± 0.71	56.59 ± 11.62
	Zircon	2138.1 ± 25.2	57.13 ± 0.62	24.78 ± 0.49	54.81 ± 4.95
	Bioplant Flora	2177.8 ± 85.3	56.72 ± 3.58	25.38 ± 1.15	57.44 ± 1.94
	AlgarenBZn	2090.6 ± 19.0	58.00 ± 0.88	20.47 ± 0.09	45.03 ± 3.31
	Average	2187.8 ± 35.6	59.57 ± 1.29	25.06 ± 0.70	52.74 ± 5.49

*The results are represented as mean ± SD (standard deviation) in four replicates.

for Mn content (only 2% of its increase) under Epin Extra treatment. Zlatno inje increased Fe, Mn and Zn content mainly in Laura grain, while Epin Extra showed the greatest impact on Mg increase in ZP-015 and Laura grains, and on Mn increase in ZP-015 and Nena grain, as well as on Fe increase in Nena grain. Zircon was the most efficient for Fe and Zn increase in ZP-015 and for Mg increase in Nena grains. Among the applied fertilizers, only Bioplant Flora was responsible for Zn content increase in Nena grains.

Availability of mineral nutrients

Variations in ratio between phytate (Phy) and β-carotene may indicate possible availability of nutrients [12,23]. This trait is an important parameter for the characterisation of the investigated genotypes. From this point, ZP-015 could be considered as a favourable variety for breeding towards an improved nutritive quality, having the lowest ratios for Phy/Mg, Phy/Fe and Phy/Mn in the control (Table 3). This indicates an additional quality of this genotype for a possible increase of mineral nutrients availability, under application of organo-mineral foliar fertilizers. This is important, since Luo and Xie [14] and Hess et al. [24] found that food rich in β-carotene can significantly enhance Fe and Zn bioavailability from grain, while either low phytate level or its degradation may enhance Mn availability [16]. Besides ZP-015, Laura variety was characterised by the lowest P_{phy}/P_i and Phy/Zn ratios. This may become a possible indicator for further P_{phy} decrease during breeding [3,22], although further research is required. When the individual impact of each foliar fertilizer on all three genotypes was considered, Zlatno inje decreased P_{phy}/P_i and Phy/Mn ratios to the largest extent, while Zircon was the most prominent for Phy/β-carotene, Phy/Mg and Phy/Zn ratios. Epin Extra was mostly efficient in Phy/Fe decrease. Since Fe and Zn deficiencies are common worldwide [5], it is very important that Zircon and Epin Extra, that are organo-mineral foliar fertilizers which are primarily dedicated to fortification, increased average Fe and Zn content in soybean grains. Furthermore, each fertilizer had a specific site of action: Zircon and Epin Extra decreased P_{phy}/β-carotene, Phy/Mg, Phy/Fe and Phy/Zn ratios in grain of ZP-015, while Bioplant Flora and Epin Extra decreased P_{phy}/β-carotene, Phy/Mg and Phy/Fe ratios in Nena grain. In Laura variety, application of Zlatno inje induced a decrease in P_{phy}/β-carotene, Phy/Fe, Phy/Mn and Phy/Zn ratios.

Different responses of the examined soybean varieties to the applied treatments can be better visualised by PC

Table 3 The effect of different foliar fertilizers on investigated ratios in grain of three soybean varieties

	Treatment	P_{phy}/P_i*	Phy/β-carot	Phy/Mg	Phy/Fe	Phy/Mn	Phy/Zn
ZP-015	Control	43.17	774.3	0.48	16.60	36.74	31.67
	Zlatno inje	33.15	865.5	0.46	15.34	35.90	23.70
	Epin Extra	40.63	709.2	0.42	14.37	32.57	28.53
	Zircon	35.44	523.6	0.46	13.01	39.57	21.20
	Bioplant Flora	33.22	641.6	0.45	15.51	39.83	23.72
	AlgarenBZn	27.61	689.9	0.45	15.71	37.36	27.99
	Average	34.77	685.7	0.45	15.03	36.84	25.69
Nena	Control	27.24	739.4	0.51	19.90	43.89	30.48
	Zlatno inje	26.87	929.5	0.50	17.42	44.91	29.70
	Epin Extra	26.41	712.2	0.48	16.22	39.70	32.51
	Zircon	44.42	833.1	0.46	16.88	45.19	29.98
	Bioplant Flora	40.19	640.8	0.45	17.02	46.79	26.62
	AlgarenBZn	40.14	835.0	0.50	18.63	49.94	35.85
	Average	32.58	770.5	0.49	17.63	44.73	30.61
Laura	Control	27.25	922.2	0.49	17.54	39.64	24.60
	Zlatno inje	30.27	827.1	0.50	15.90	38.50	17.61
	Epin Extra	25.71	865.8	0.48	17.36	39.92	18.21
	Zircon	25.78	750.5	0.45	17.67	40.73	18.41
	Bioplant Flora	25.31	973.9	0.48	18.45	41.23	18.22
	AlgarenBZn	26.14	842.1	0.47	18.12	51.34	23.34
	Average	26.66	858.5	0.48	17.47	41.52	19.73

*Ratios: P_{phy}/P_i, phytic and inorganic P; Phy/β-carot, phytate and β-carotene; Phy/Mg, Phy/Fe, Phy/Mn, Phy/Zn, phytate and mineral nutrients.

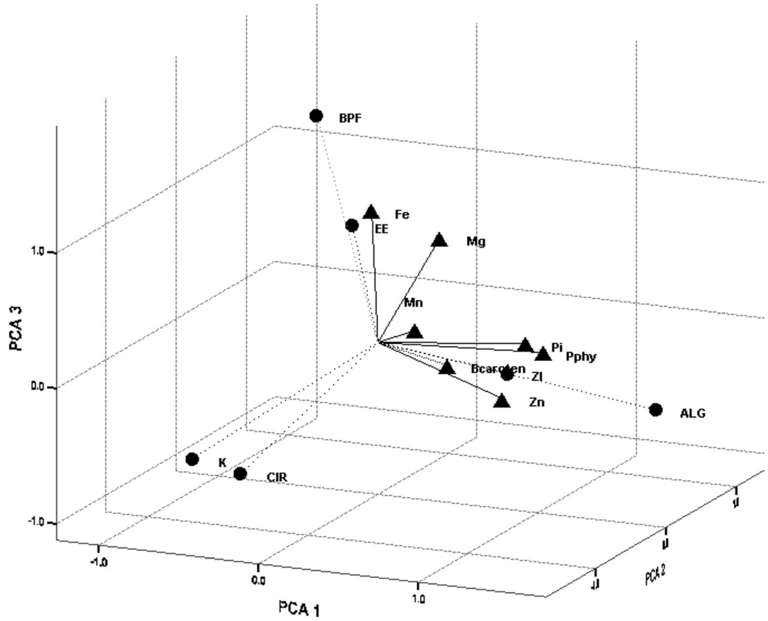

Figure 1 **Principal component analysis (PCA) for chemical composition of grain in ZP-015 variety (P_{phy}, phytic phosphorus; P_i, inorganic phosphorus, K, control; ZI, Zlatno inje; EE, Epin Extra; CIR, Zircon; BPF, Bioplant Flora; ALG, Algaren BZn).**

analysis. In grains of ZP-015, results indicated the Epin Extra treatment as the most efficient for Fe and Mg content increase (Figure 1). Positive effects of Zlatno Inje and Algaren BZn were shown mostly on the increase of P_i and P_{phy} content, and, partially, on β-carotene and Zn content. In Nena's grain, a most pronounced increase was found in β-carotene, Mg and Fe contents under the application of the Zircon treatment, thus positively affecting the bioavailability of these mineral elements (Figure 2). However, application of investigated foliar fertilizers did not demonstrate the expected positive effect on an improved content and bioavailability of Zn. In Laura's grain, Epin Extra was the most efficient foliar fertilizer, leading to an increase in P_{phy}, Fe and Zn

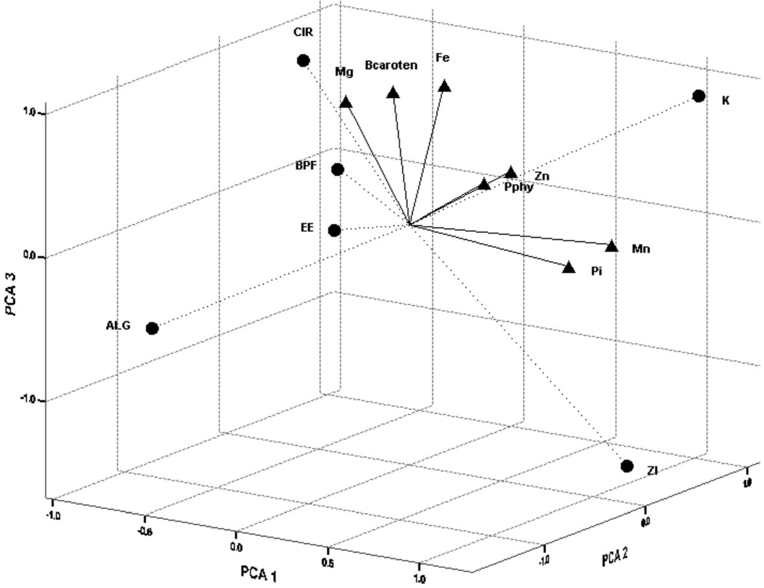

Figure 2 **Principal component analysis (PCA) for chemical composition of grain in Nena variety (P_{phy}, phytic phosphorus; P_i, inorganic phosphorus, K, control; ZI, Zlatno inje; EE, Epin Extra; CIR, Zircon; BPF, Bioplant Flora; ALG, Algaren BZn).**

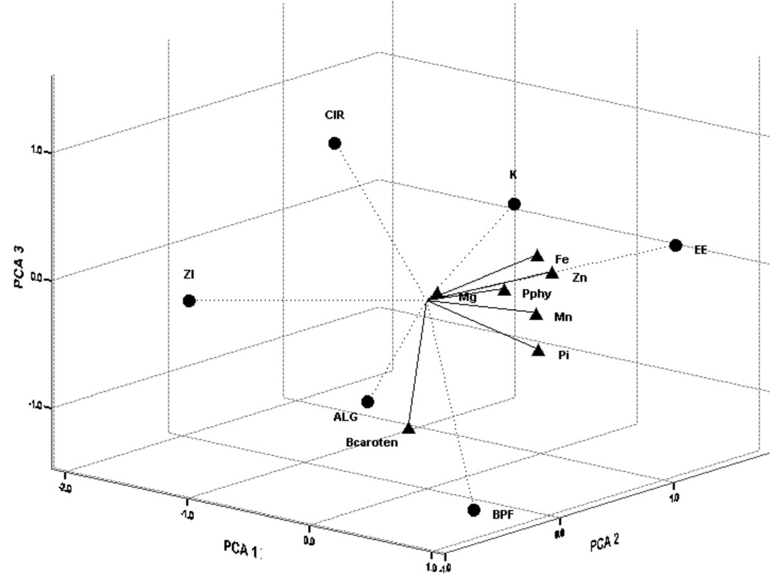

Figure 3 Principal component analysis (PCA) for chemical composition of grain in Laura variety (P_{phy}, phytic phosphorus; P_i, inorganic phosphorus, K, control; ZI, Zlatno inje; EE, Epin Extra; CIR, Zircon; BPF, Bioplant Flora; ALG, Algaren BZn).

content, and, by a lower extent, to that in P_i and Mn content, whereas treatment with Algaren BZn showed a β-carotene content increase (Figure 3). Such results suggest that measures to increase mineral nutrients in soybean grain (such as fortification) were related to alterations of P_{phy} and β-carotene. Moreover, results obtained on maize and beans did not support a lower Fe availability from grains linked to slightly larger phytate content, while a significant effect on Fe availability is commonly atributed to promoting factors [25].

Interactions between examined parameters may be important for understanding potential availability of mineral elements. Results presented in Table 4 indicated that a large grain weight correlated significantly and positively with Zn content but negatively with β-carotene. Conversly, significant and negative correlation between P_{phy} and Zn may be directly related to an improved Zn availability, as shown by the results of Lönnerdal [3] and Underwood and Suttle [13], who ascertained that any reduction in phytic acid content in food is likely to result in improved Fe, Zn and Mn status. Improved Fe availability could be supported by its significant and positive correlation with β-carotene. Significant and positive correlation between Mg, Fe and Mn, as well as with P_{tot} indicates that an improved mineral nutrition under application of organo-mineral foliar fertilizers, may increase the concentration of individual elements in grain, althogh their availability may be still questionable. Opposite to Mg, Fe and Mn, the Zn increase was primarily related to a yield parameter, such as the 1,000 grains weight, that was mainly increased by Bioplant Flora (Table 1).

Table 4 Correlations between investigated parameters in grain of three soybean varieties

	1,000 grain weight	P_{tot}**	P_i	P_{phy}	β-carot.	Mg	Fe	Mn
P_{tot}	−0.310*							
P_i	0.367	−0.394						
P_{phy}	−0.370	−0.063	−0.052					
β-carot.	−0.596*	0.475*	−0.377	−0.016				
Mg	−0.264	0.674*	−0.601*	0.091	0.389			
Fe	−0.359	0.801*	−0.513*	−0.233	0.654*	0.581*		
Mn	−0.066	0.660*	−0.179	−0.015	0.135	0.651*	0.571*	
Zn	0.683*	−0.256	0.389	−0.494*	−0.203	−0.204	−0.082	0.006

*Correlation is significant at 0.05 level.

**P_i, inorganic P; P_{phy}, phytic P; P_{tot}, total P.

Conclusions

Our findings showed that ZP-015 can be generally considered as a favourable variety for increased bioavailability of Mg, Fe and Mn (due to the lowest ratios of Phy/Mg, Phy/Fe and Phy/Mn found in control samples). Moreover, in all varieties, an improvement in grain yielding potential and grain quality was achieved through foliar application of organo-mineral fertilizers. Positive effect of Zircon application was evident on the increased grain weight, and β-carotene and Fe content. The latter which may, along with the lowest values obtained for Phy/β-carotene and Phy/Mg ratios, have determined an increase in Mg and Fe bio-availability, mainly for Nena grain. On the other hand, positive effect of Epin Extra was observed mostly because of both Phy decrease and Fe and Mn increase, thus contributing to their increased bio-availability. Accordingly, organo-mineral foliar fertilizers based mainly on phenolic acids (Zircon) and bioregulators (Epin Extra) should be recommended to be used for soybean fortification.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Bogdan Nikolić and Vesna Dragičević designed the research. Bogdan Nikolić, Vesna Dragičević, Hadi Waisi, Igor Spasojević and Vesna Perić performed field trials. Milovan Stojiljković, Vesna Dragičević and Sanja Đurović performed chemical analyses. Vesna Dragičević and Igor Spasojević analyzed the data. Vesna Dragičević and Bogdan Nikolić wrote the paper. All authors read and approved the final manuscript.

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