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BARLEY GRAIN ENRICHEMENT WITH ESSENTIAL ELEMENTS BY AGRONOMIC BIOFORTIFICATION

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Barley grain is rich in mineral nutrients, but their bioavailability to humans depends on antinutrients that restrain bioavailability and promoters that promote bioavailability. The aim of this study was to examine composition of barley grain, including phytate and phenolics as antinutrients, carotenoids and glutathione as promoters and mineral elements, such as Ca, Mg, Fe, Si, Zn and Mn influenced by various non-standard foliar fertilizers (Zircon, Chitosan, Siliplant, Propikonazole), including some hormonal growth-stimulators (Epin Extra, Benzyladenine), as potential biofortification measure. Chitosan increased glutathione concentration in grain. Unfavorable meteorological conditions were partly mitigated by application of Benzyladenine and Siliplant, reflected through increased potential bioavailability of P, Mg, Ca and Fe.

KEY WORDS: barley grain composition, antioxidants, mineral elements, biofortification

INTRODUCTION

Wholegrain products are necessary part of healthy diets as sources of dietary fiber and mineral nutrients. Among cereals, barley grain is the main source of P, Ca, K, Mg, Na, Cu and Zn (1), as well as of Si, which showed the positive effect on bones (2). Barley health benefits are provided by a β-glucan fiber fraction, which is associated with lowering of blood cholesterol levels, glycemic index and weight loss (3).

High concentration of mineral elements in cereal grains does not mean that they are available for humans. Antinutrients, as essential part of grain, like phytate, phenolics, etc., limit the absorption of mineral elements. Grains also contain promoters, such as β-carotene, S-containing amino acids, etc., which enhance mineral nutrients bioavailability or decrease the activity of inhibitors (4, 5). Enrichment of grains with mineral elements and other important nutrients – biofortification is a very complex process. One of the strategies is the application of foliar fertilizers, which have also a positive effect on plant metabolism and grain yield (6).

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There are many different substances which foliarly applied influence accumulation of mineral nutrients in grains. For instance, Si can improve crop production by increasing water uptake, maintaining nutrient balance, promoting photosynthetic rate, increasing the activities of antioxidants (7, 8). Nevertheless, hormones could also increase stress tolerance and uptake of mineral nutrients, like brassinosteroids (9). Propiconazole, as a brassinosteroid inhibitor (10) showed positive impact on barley and wheat grain yield and composition, decreasing phenolics and increasing carotenoids (11). Cytokinine, 6-benzyladenine expressed positive effect on photosynthesis and antioxidants in salt stressed plants (12). Chitosan has ability to mitigate stress and to chelate minerals and other nutrients, and it is widely used in phytoremediation for heavy metal removal (13).

The aim of this study was to test various foliar fertilizers, including some hormonal growth-stimulators, as potential biofortification measure on chemical composition of barley grain, including phytate and phenolics as antinutrients, β-carotene and glutathione as promoters and mineral elements, such as Ca, Mg, Fe, Zn and Mn.

EXPERIMENTAL

Field trial

The grain of hull-less barley (*Hordeum vulgare* L. *var. nudum*; cv. "Apolon") was produced in 2013 and 2014 at Zemun Polje (44^o52'N, 20^o20'E; 86 ± 3 m altitude). The experiment was set up in four replications. Foliar fertilizers were applied in recommended concentrations. Treatments included 0.335 ml L^{-1} of Epin Extra (24-epibrassinolide phytohormone with 0.025 g a.i. L^{-1}); 0.3 ml L^{-1} of Zircon (*Echinacea purpurea* extract, with 0.1 g L^{-1} of phenolic acids: 3,4-dihydroxycinnamic (caffeic) acid, chlorogenic and cichoric acid); 1 ml L^{-1} of Chitosan (0.5% of polysaccharide chitosan, 3-4% of organic C, 2-5% of organic N, 5% of amino acids and 10% of humic acids); 2 ml of 6-benzyladenine (technical grade 90%, syn. 6-BA or BAP); 3 ml of Siliplant (16.9±2.0 g L^{-1} of K, 0.13±0.05 g L^{-1} of Mg, 72.0±4.0 g L^{-1} of Si, 0.45±0.1 g L^{-1} of Fe, 0.32±0.09 g L^{-1} of Mn, 0.12±0.04 g L^{-1} of B, 0.08±0.03 g L^{-1} of Zn, 0.07±0.03 g L^{-1} of Cu and 0.02±0.01 g L^{-1} of Co); 0.2 ml of Propikonazole (PZR, technical grade 95%); control – without application of foliar fertilizers. First spraying for all treatments, except for BAP and PZR was performed 45 days after emergence, while the second one was 60 days after emergence (when BAP and PZR were applied).

Chemical analysis of barley grain

After harvesting the chemical composition of grain was determined. Phytic (P_{phy}) and inorganic (P_i) phosphorus were determined by the method of Dragičević et al. (14), and total glutathione (GSH) by the method of Sari Gorla et al. (15), after extraction with 5% trichloroacetic acid. The extract was centrifuged at 12,000 rpm for 15 min (Dynamica – Model Velocity 18R Versatile Centrifuge, Rotor TA15-24-2) at 4^oC. P_{phy} was determined on a Biochrom Libra S 22 spectrophotometer, based on the pink color of the Wade reagent, which is formed upon the reaction of ferric ion and sulfosalicilic acid, and has an absorbance maximum at λ=500 nm. P_i was determined after adding of ammonium heptamolybdate + ammonium metavanadate solution to the extract and measuring the absorbance at λ=400 nm. GSH was determined by adding 0.2 M potassium phosphate buffer (pH = 8.0) and 10 mM DTNB (5.5'-dithio(2-nitrobenzoic acid)) to the extract and measuring the absorbance at 415 nm. Water soluble phenolics were determined by the method of Simić et al. (16), after extraction with double distilled water and centrifugation at 12000 rpm for 15 min, by adding 0.05 M $FeCl_3$ in 0.1 M HCl and 0.008 M $K_3Fe(CN)_6$ to sample solution; the absorbance was measured at λ=722 nm. Phenolic content was expressed in μg of ferulic acid (FAE) equivalent. Yellow pigment (β-carotene) concentration was determined according to the AACC procedure (17), after extraction with 1-butanol and centrifugation at 10,000 rpm for 5 min; the absorbance was measured at λ=436 nm.

After wet digestion with HNO_3 + $HClO_4$, the Ca, Mg, Fe, Mn, Zn and Si contents were determined by Inductively Coupled Plasma - Optical Emission Spectrometry (Spectro Analytical Instruments, Germany).

Statistical analysis

Chemical composition of the barley grain is present as mean ± standard deviation (SD). The ratios between P_{phy} and P_i, phytate, β-carotene, Ca, Mg, Fe, Zn and Mn could be considered as parameters of potential bioavailability of examined mineral elements and the differences between treatments means were tested by ANOVA, with Fisher's least significant difference (LSD) test at the 0.05 probability level.

RESULTS AND DISCUSSION

Meteorological conditions

Meteorological conditions had opposite trend in two experimental years. The difference in temperature between the seasons was negligible (Table 1), with the amount of precipitation being almost 2.5 times higher in 2014 than in 2013, that is near a 20-year average. The highest value of precipitation was obtained in May for both 2013 and 2014 (102.1 and 286.7 mm, respectively).

Table 1. Average monthly air temperatures and precipitation sums from March to July for 2013, 2014 and period 1991-2010, at Zemun Polje

Month		March	April	May	June	July	Average/Σ
T average (°C)	2013	7.3	14.8	19.5	21.3	24.1	17.4
	2014	11.3	14.1	17.0	21.5	23.3	17.5
	1991-2010	7.9	12.4	17.8	21.0	22.8	16.4
Σ precipitation (mm)	2013	95.5	21.7	102.1	49.8	2.7	271.8
	2014	48.6	85.4	286.7	59.5	250.0	730.2
	1991-2010	44.9	48.5	52.5	83.7	63.8	293.4

Antioxidants in barley grain. Negative conditions present during 2014 affected concentrations of main antioxidants: yellow pigments, GSH and phenolics (Figure 1). Several times higher concentrations of yellow pigment and almost twice higher concentration of GSH and phenolics in 2013, compared to 2014 were obtained. The highest values of yellow pigment and GSH were in Chitosan treatment in 2014 (0.48 µg g^{-1} and 514.8 nmol g^{-1}, respectively), and in 2013 insignificantly higher in control (4.39 µg g^{-1} and 930.5 nmol g^{-1}, respectively). Phenolics had the highest value in Chitosan and in PZR treatment, in 2013 (2275.1 µg g^{-1} and 2325.0 µg g^{-1}, respectively) and in Siliplant and PZR treatments in 2014 (1758.9 µg g^{-1} and 1799.9 µg g^{-1}, respectively).

Concentrations of P_{phy} and P_i also showed differences among experimental seasons, with slightly higher P_{phy} and lower P_i values obtained in 2013, compared to 2014 (Figure 2). Slight variations among treatments indicated that higher P_{phy} values were obtained in control of both years (4.56 and 4.22 mg g^{-1} in 2013 and 2014), while the lowest values were in Chitosan treatment (4.17 and 3.93 mg g^{-1}, respectively). Epin Extra increased P_i values in 2013 and 2014 (0.86 and 1.05 mg g^{-1}, respectively). Brassinosteroids expressed the increased activity of enzymatic and nonenzymatic antioxidants, subsiding the harmful effect of salinity and heavy metal stress in bean plants (18).

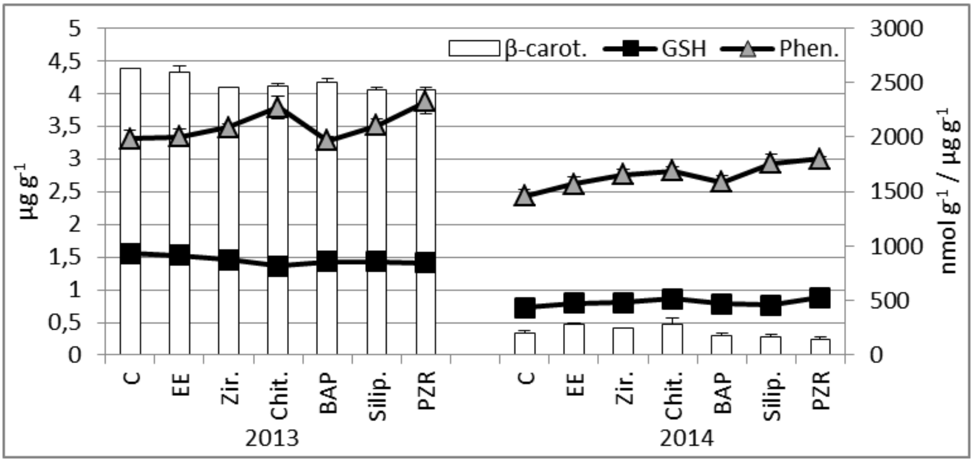

Figure 1. The effect of different foliar fertilizers on concentration of yellow pigment (β carot.), glutathione (GSH) and phenolics in barley grain (C – control, EE – Epin Extra, Zir. – Zircon, Chit. – Chitosan, BAP – Benzyladenine, Silip. – Siliplant, PZR – Propikonazole); Mean ± SD (standard deviation)

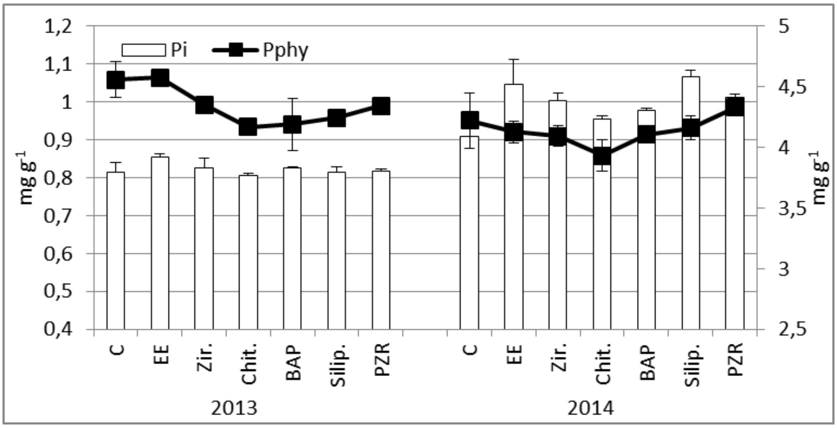

Figure 2. The effect of different foliar fertilizers on concentration of phytic (P_{phy}) and inorganic (P_i) phosphorus in barley grain (C – control, EE – Epin Extra, Zir. – Zircon, Chit. – Chitosan, BAP –Benzyladenine, Silip. – Siliplant, PZR – Propikonazole); Mean ± SD (standard deviation)

Mineral elements in barley grain. Meteorological conditions have also influenced accumulation of mineral elements in grain. This was especially true for Si, whose concentration was significantly (five times) lower in 2014 (Figure 3), and was opposite to the results of Ma et al. (2), who did not find significant impact of the year on Si variation in barley. Higher variations were observed among treatments in Ca concentration in 2014 compared to 2013.

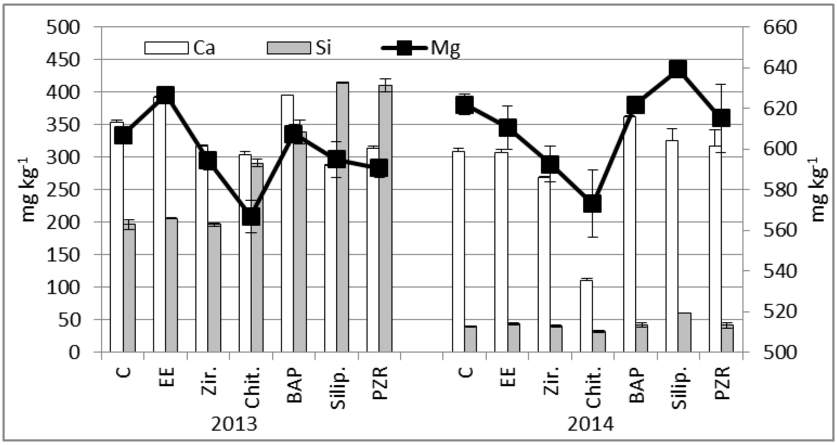

Figure 3. The effect of different foliar fertilizers on Ca, Mg and Si concentration in barley grain (C – control, EE – Epin Extra, Zir. – Zircon, Chit. – Chitosan, BAP – Benzyladenine, Silip. – Siliplant, PZR – Propikonazole); Mean ± SD (standard deviation)

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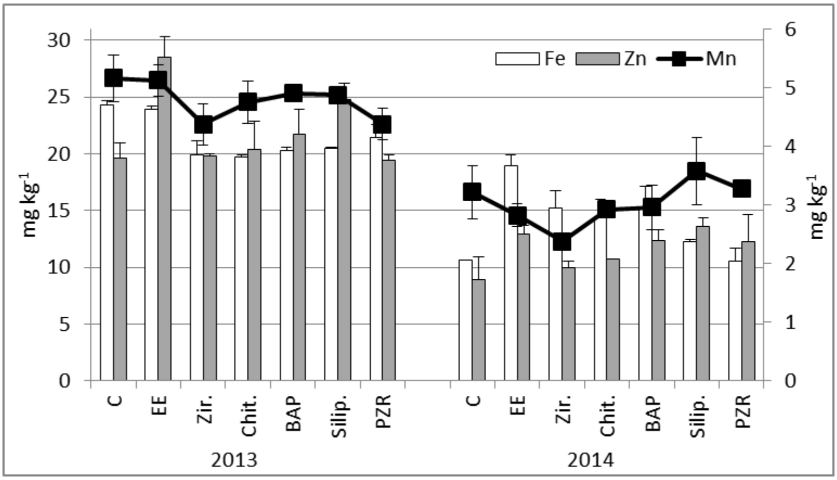

Figure 4. The effect of different foliar fertilizers on Fe, Zn and Mn concentration in barley grain (C – control, EE – Epin Extra, Zir. – Zircon, Chit. – Chitosan, BAP – Benzyladenine, Silip. – Siliplant, PZR – Propikonazole); Mean ± SD (standard deviation)

Epin Extra highly influenced Mg concentration, similarly to the results of Lachman et al. (19), as well as the concentrations of microelements, Fe, Zn and Mn (Figure 4), similarly to the results of Dragičević et al. (20), but under favorable meteorological conditions. Besides, higher Ca concentrations were observable in BAP treatment for 2013 and 2014 (395.0 and 362.8 mg kg^{-1}, respectively), according to the results of Gurmani et al. (21), who obtained higher Ca concentrations in wheat plants treated with BAP under saline and non-saline conditions. Furthermore, Siliplat was the most important fertilizer for Si increase in barley grain (414.7 and 60.4 mg kg^{-1}, for 2013 and 2014), as well as for Zn and Mn increase during unfavorable, 2014 (13.59 and 3.58 mg kg^{-1}, respectively), confirming that Si plays an important role in osmotic adjustment and regulation of the hormonal plant status under stress conditions (8).

Table 2. The effect of different foliar fertilizers on the relations between phytic and inorganic P, phytate, yellow pigment, Mg, Ca, Fe, Zn and Mn in barley (cv. Apolon) grain

Treatment	P_{phy}/P_i	Phy/yell.pigm.	Phy/Mg	Phy/Ca	Phy/Fe	Phy/Zn	Phy/Mn
Control	5.10 $^{**n.s.}$	5356.60 $^{n.s.}$	2.15 ^{b}	2.86 ^{ab}	107.34 ^{b}	40.22 $^{n.s.}$	74.1 ^{b}
Epin extra	4.58 $^{n.s.}$	5242.48 $^{n.s.}$	2.11 ^{ab}	2.68 ^{ab}	100.90 ^{b}	27.52 $^{n.s.}$	60.0 ^{a}
Zircon	4.62 $^{n.s.}$	5411.22 $^{n.s.}$	2.14 ^{a}	3.11 ^{b}	103.15 ^{b}	37.21 $^{n.s.}$	71.0 ^{ab}
Chitosan	4.60 $^{n.s.}$	5088.97 $^{n.s.}$	2.14 ^{a}	4.21 ^{c}	72.38 ^{a}	34.10 $^{n.s.}$	70.1 ^{ab}
Benzyladenine	4.60 $^{n.s.}$	5349.25 $^{n.s.}$	2.03 ^{a}	2.36 ^{a}	62.91 ^{a}	31.81 $^{n.s.}$	69.1^{ab}
Siliplant	4.47 $^{n.s.}$	5610.72 $^{n.s.}$	2.05 ^{ab}	2.96 ^{b}	51.13 ^{a}	28.70 $^{n.s.}$	76.1 ^{b}
Propikonazole	4.74 $^{n.s.}$	5828.46 $^{n.s.}$	2.16 ^{b}	2.96 ^{b}	55.46 ^{a}	35.80 $^{n.s.}$	80.3 ^{b}
LSD 0.05*	0.8	2397.6	0.11	0.58	26.27	15.66	10.43

* Least significant difference, P = 0.05 (n = 4); The values followed by same letters are not significantly different at the 0.05 level; $^{**n.s}$The values are under the level of significance of 0.05.

The highest Fe concentration in barley grain was observed in Epin Extra treatment (23.9 and 18.9 mg kg^{-1}, for 2013 and 2014). Insignificantly higher Fe and Mn concentrations in control plants were obtained in 2013, due to the drought present during the grain filling period, similarly to the results of Hussein et al. (22) who also found lower concentrations of both elements in grain of barley sprayed with amino acid fertilizers in combination with water deficit. It could be supposed that foliar treatments, irrespective of the formulation applied, slightly reduced accumulation of some promoters, such yellow pigment and GSH (Figure 1), as well as some mineral elements (Fe and Mn; Figure 4) in dry conditions. However, positive impact of applied fertilizers on accumulation of examined elements was mainly related to hormone preparations, such as EE and BAP, as well as silicone fertilizer Siliplant, illustrating their complex role in plant metabolism and stress tolerance (8, 9, 12), which can also reflect on increased nutrients acquiring in barley grain.

Potential bioavailability of mineral elements. Considering the relations between Phy, P_i, yellow pigments, Mg, Ca, Fe, Zn and Mn, the highest values were mainly obtained in PZR treatment, with significant differences observed between the values of Phy/yellow pigment, Phy/Mg, Phy/Ca, Phy/Fe and Phy/Mn (Table 2). The lowest values of Phy/Zn and Phy/Mn were in hormone, Epin Extra treatment (25% and 23% lower in relation to PZR, respectively), while the significantly lowest values of Phy/Mg and Phy/Ca were in BAP treatment (6% and 44% lower in relation to PZR, respectively). Siliplant was characterized by the significantly lowest values of Phy/Fe in relation to control (12% and double, respectively) and Chitosan with significantly lowest Phy/yellow pigment (13% in relation to PZR). While Chitosan mainly decreased P_{phy} concentration, the increased concentrations of examined mineral elements influenced by Siliplant and hormone preparations Epin Extra and BAP were mainly reflected on decrease of the ratio between phytate and mineral elements, contributing to their better bioavailability to humans (4).

CONCLUSION

The obtained results indicate that the year of cultivation influenced the chemical composition of barley grain, mainly increasing concentrations of promoters, antinutrients and mineral elements during dry season. The highest impact of unfavorable conditions with high precipitation level was for Si, decreasing its concentration several times. Among applied treatments, Chitosan was the most effective for increasing of promoters' level, and reducing of Phy/yellow pigment ratio, thus increasing potential bioavailability of the examined mineral elements. Moreover, unfavorable meteorological conditions were partially mitigated by application of Siliplant and hormone preparations: EE and BAP, thus increasing potential bioavailability of P, Mg, Ca and Fe.

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ОБОГАЋИВАЊЕ ЗРНА ЈЕЧМА ЕСЕНЦИЈАЛНИМ ЕЛЕМЕНТИМА ПУТЕМ АГРОНОМСКЕ БИОФОРТИФИКАЦИЈЕ

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Зрно јечма је богато минералима, али њихова приступачност за људски организам зависи од антинутритива који инхибирају њихову апсорпцију и промотера који повећавају њихову приступачност. Циљ истраживања је да се испита састав зрна јечма, укључујући фитат и феноле као антинутритиве, каротеноиде и глутатион као промотере, као и минералне елементе Ca, Mg, Fe, Si, Zn и Mn, под утицајем нестандардих фолијарних ђубрива (Циркон, Хитосан, Силиплант, Пропиконазол), као и хормона (Епин Екстра, Бензиладенин), као потенцијалне мере за биофортификацију јечма. Хитосан је повећао концентрацију глутатиона. Неповољни метеоролошки услови су делимично превазиђени применом Бензиладенина и Силипланта, који су утицали на повећање потенцијалне приступачности P, Mg, Ca i Fe.

Кључне речи: хемијски састав зрна јечма, антиоксиданти, минерални елементи, биофортификација

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