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Bioavailability

Effect of nitrogen and zinc fertilization on zinc and iron bioavailability and chemical speciation in maize silage

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ABSTRACT

Agronomic biofortification is one of the main strategies for alleviation of micronutrient deficiencies in food and feed. The objective of this study was to investigate the effect of N supply on total concentration of Zn and Fe and their chemical species in the soluble extracts of maize silage grown under field conditions. Total concentrations of Zn, Fe, Cu, Mn, S and P were measured by flow-injection inductive coupled plasma (ICP) – mass spectrometer (MS). Soluble Fe and Zn were extracted and analyzed by size exclusion – inductively coupled plasma mass spectrometry. Using the same set-up for total elemental and speciation analysis enabled direct quantitative comparison of the detected speciated molecules with the total element sample content. N or Zn treatment, except in control plots, did not significantly affect concentrations of Zn and Fe in the maize silage and grain samples. Significant positive correlation was observed between Zn and Fe maize silage ($r = 0.64$, $p < 0.01$) and maize grain ($r = 0.85$, $p < 0.01$) concentrations. N and Zn treatment did not affect solubility of Zn and Fe, while available Zn and Fe were affected by increase in Zn soil treatment. Soluble Zn was speciated in LMW complexes, while soluble Fe was speciated in MMW and LMW complexes.

1. Introduction

Micronutrient deficiency creates serious human health problems, largely in countries of the developing world, but it also negatively affects productivity of farm animals grown in these countries [1–3]. It is reported that more than two billion people worldwide (Asia, Africa and Latin America), mostly children and pregnant women whom suffer from severe malnutrition, are affected by zinc (Zn) and iron (Fe) deficiency [4,5]. Zinc and Iron are essential elements for normal growth and development of farm animals [6,7]. Both elements play very important metabolic role as activators of different enzymes in animal body [8].

Maize (Zea mays L.) is one of the most grown field crops in the world and 67% of globally produced maize is used as livestock feed source either as grain or as silage [9].

Agronomic biofortification is recognized as an effective tool in alleviating the micronutrient deficiency in plants, where nitrogen (N) supply is considered as an important factor in enhancing the concentrations of Zn and Fe in the crops [10].

The speciation of Zn and Fe is also an important factor in determining grain and feed nutritional quality. Zinc and Fe are present in plants in various chemical species, which may vary in their bioavailability. Recent research indicates that complexes of Zn and Fe with nonprotein amino acid nicotianamine (NA) and 2′-deoxymugineic acid (DMA) are present in cereals [11], indicating that they may play a role in Fe and Zn storage. Most of the Zn, Fe, and other minerals in cereal grain are complexed by phytic acid, inositol hexakisphosphate (IP_6), resulting in salts known as phytates, which may be insoluble or soluble depending on the degree of metal bonding to phosphate groups [12,13]. In addition, some of the soluble Fe in cereal grains is bound to inositol pentaphosphate (IP_5), a dephosphorylated soluble form of IP_6 [14,15]. If increasing N supply increases the transport of NA- or DMA-chelated forms of Fe and Zn in cereal crops like wheat, barley and rice, one may expect increased levels of these complexes in maize as well, considering that thy are same strategy II group plants [16]. However, speciation of these nutrients in animal feed and especially silage, containg both leafy material and grain, is not very well known.

The objective of this study was, therefore, to investigate the effect of N supply on total concentration of Zn and Fe and their chemical species in the soluble extracts of maize silage grown under field conditions.

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2. Materials and methods

2.1. Field experiment

A maize (Zea mays L.) field experiment was conducted in Northwest Bosnia and Herzegovina (45° 06′ 28″ N; 16° 46′ 21″ E) in a completely randomized block design with four replicated plots (2 × 3 m). The soil type of experimental field was fluvisol with pH of 6.4. Soil contained 3.02 mg/kg and 359 mg/kg of extractable Zn and Fe (extracted by Mehlich-3 (M3) [17]), respectively. Precipitation ranged from 78.4–237 mm and the minimum and maximum temperature were 13.3–21.8 °C during the course of this field experiment.

The experiment consisted of control and two levels each of N and Zn fertilizers. Nitrogen rates were 120 kg/ha and 180 kg/ha defined as lower and higher levels. Nitrogen was applied in split application with 50% one month before sowing in the form of complex NPK fertilizer and urea, and the rest 50% at 7–8 leaf stage of maize growth in the form of calcium ammonium nitrate. Zinc rates were 12.5 kg/ha as lower Zn and 18.75 kg/ha as higher Zn in the form of $ZnSO_4 \times 7H_2O$. Zinc was applied before sowing of maize crop. Zinc fertilizer solution, ZnSO4 × $7H_2O$ dissolved in 5 Liters of distillated water, was sprayed on the soil surface. Soil was tilled using roto cultivator prior to sowing. Phosphorus at the rate of 70 kg/ha P_2O_5 and K at the rate 130 kg/ha K_2O were applied to all plots in the form of complex NPK fertilizers one month before sowing.

Maize was sown in rows with row distance of 70 cm and plant distance of 20 cm. Chemical weed control treatment was applied to all plots after sowing and before emerging of the maize. Additional fertilization of N was applied between rows at 7–8 leaf stage of maize growth.

Maize plants for silage were harvested at milky-wax maturity stage of grain. Ten plants from middle rows were harvested from each plot. Plants were cut at 20 cm above the ground to avoid sample contamination with soil. Harvested plants were chopped using stationary forage cutter and put into plastic bags for fermentation for two months. Plastic bags were pressed to remove as much air as possible before sealing them tightly using rope and plastic warp. After two months, bags were opened to perform sampling of maize silage. Collected silage samples were put in vacuum bags to keep them fresh, prior to drying. Silage samples were dried at 120 °C until constant weight was observed. Dried silage samples were milled prior to their use in chemical analysis and speciation studies. In addition to maize silage, grain samples from the same plots as the silage maize samples were also collected. Similar to sampling of silage maize, 10 plants in the middle rows were harvested for grain sampling. Grain samples were dried and milled before chemical analysis.

2.2. Chemical analysis for total nutrient concentration

Dried and milled grain and silage samples were subject to acid digestion. 200 mg of sample in a mixture containing 5 mL HNO_3 (69%, v/v) and 2.5 mL H_2O_2 (30%, v/v) were put in a pressurized microwave digestion system (UltraWAVE; Milestone Srl, Sorisole, Italy). After the digestion, samples were diluted to a final concentration of 3.5% HNO_3 using Milli-Q water [18]. The digested solutions were analyzed for total concentration of Zn, Fe, Cu, Mn, S and P by flow-injection inductive coupled plasma (ICP) – mass spectrometer (MS) using an HPLC (Ultimate 3000) equipped with an ICS 5000 pump (Thermo Scientific), as an auto-sampler. The transient signals was quantified on an Agilent 8800 triple quad, Agilent Technologies. The qqq-ICP-MS is equipped with an octopole reaction cell were the reaction gas, oxygen was used to move the critical elements S, P and Fe away from polyatomic interference. Using this methodology simultaneous trace analysis of ^{32}S (as $^{48}SO+$), ^{56}Fe (as $^{72}FeO+$), ^{31}P (as $^{47}PO+$), ^{66}Zn, ^{63}Cu, and ^{55}Mn was possible. Data was processed using the Masshunter 4.2 (Agilent Technologies) software package. For the specific setup of FIA-ICP-MS analysis using oxygen as a reaction gas the limit of detection (LOD) and limit of quantification (LOQ) was calculated based on calibration points close to LOD/LOQ using the formula LOD = 3.3(SD response/slope) and LOQ = 10(SD response/slope). This gave an LOD of 2.57 ppb for Zn and 3.54 ppb for Fe. The LOQ was 7.79 ppb and 10.73 ppb respectively.

2.3. Size exclusion chromatography – ICP-MS analysis

For speciation analyses, 50 mg of silage sample was extracted with 7 mL of degassed 50 mM TRIS-HCl buffer (pH 7.5) and 300 mg of acid-washed quartz sand. The extractions were performed over a period of 60 min using an ice-cold mortar and a pestle. The pestle was used every 15 min during the incubation period to homogenize the sample. After 60 min, the samples were centrifuged (16 000 − × g, 2 °C, 15 min). The supernatant was ultra-filtered with a 100 kDa ultra filter (Amicon 100; Millipore) prior to analysis. The analytical size exclusion chromatography (SEC) column was a Biobasic SEC 120 (300 9 7.8; 5 Lm particle size, 120 Å pore size; Thermo Fisher Scientific, Waltham, MA, USA) mounted with a pre-column (Biobasic SEC 120 pre-column; Thermo Fisher Scientific). The Biobasic SEC column was composed of deactivated 5 µm silica coated with a hydro-link polymer to reduce the impacts of secondary ionic or hydrophobic interactions. Chromatography was performed using the same set-up as for total elemental quantification (UltiMate 3000 and ICS 5000, Thermo Scientific). All connections were of PEEK material with an internal diameter of 150 Lm (nano Viper, Dionex; Thermo Fisher Scientific). After separation on the Bio Basic column first detection was done on a connected diode array detector (DAD) were (not making sense here) after elemental detection and quantification was performed on the qqq- 8800 Agilent ICP-MS operated in oxygen mode, as described above. The SEC-column was size-calibrated with the following mixture of standard metallo-proteins; ferritin (Fe) (382 kDa), superoxide dismutase (Cu/Zn) (32 kDa), myoglobin (Fe) (17 kDa) and vitamin B12 (Co) (cyanocobalamin; 1.3 kDa), all purchased from Sigma-Aldrich.

Using the same set-up for total elemental and speciation analysis enabled direct quantitative comparison of the detected speciated molecules with the total element sample content. The concentrations of Fe and Zn bound to the individual peaks in the Tris-HCl-extractable fractions were estimated by multiplying the relative peak areas by the soluble concentrations of these elements in the extracts. As Tris − HCl provided a pH buffer only, Tris − HCl-extractable Fe and Zn can be considered to represent the water-soluble fraction. Therefor insoluble Fe and Zn concentrations were estimated by the difference between the total and soluble concentrations.

2.4. Statistical analysis

Statistical analysis was performed by using R commander program (version 3.2.5). The significance of the effects of treatments and their interactions on the reported traits was evaluated by analysis of variance (ANOVA). Significant difference among means was determined by using the Tuckey test at 5% level ($P \leq 0.05$), whenever ANOVA (using general linear model) indicated significant effect of treatments. Pearson's correlation test was used to test correlation significance.

3. Results

3.1. Concentrations of Zn and Fe in maize silage and maize grain

Fig. 1 shows the concentrations of Zn and Fe in the maize silage samples grown in the field experiment in Bosnia and Herzegovina. There was no significant difference in Zn maize silage concentration between two Zn soil treatments (Zn lower, Zn higher), while N (N higher) treatment showed positive effect only for control treatment (Fig. 1A). Either N or Zn supply did not significantly affect iron concentration in maize silage. Although, significant positive correlation