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Assessing Crude Protein Levels and Microelement Concentrations of Flour Grain Yield of *Sorghum bicolor* (L.) Moench Cultivars

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Sorghum bicolor (L.) Moench is scientifically known as a highly adaptable cereal crop with a high nutritional value, including significant amounts of crude protein and trace components. Crude protein encompasses various amino acids essential for growth, development, and maintaining quality. Trace elements play a crucial role in metabolism and activating enzymes. By examining the levels of crude protein and trace elements in various Hungarian sorghum cultivars, the research aims to show how nitrogen's impact varies across different cultivars. The assessment research focused on six chosen cultivars: Zádor, Alföldi 1, ES Albanus, Albita, and Farmsugro180. These types displayed different hues, including red, red/brown, and white. The cultivar ES Föehn, developed by Lidea Seeds, served as the benchmark for European standards. The elements N, Fe, Zn, Cu, and Sr were selected for the intended investigation. The treatment consisted of a control group that did not get any nitrogen fertilizer and a treated group that received nitrogen fertilizer in the form of ammonium nitrate (Péti-só, 27% N). The treated group was managed with a 60 $kg \cdot ha^{-1}$ dosage in the experimental field. The findings revealed variations in the concentrations of elements depending on the different kinds within each category. Most of the analyzed groups exhibited a statistically significant *p*-value of less than 0.05, as determined by the variance of non-parametric data for Fe, Cu, Zn, and Sr. For example, Zádor exhibited abundant microelement contents, such as N (15.3 $mg \cdot kg^{-1}$), Fe (46.2 $mg \cdot kg^{-1}$), Cu (3.5 $mg \cdot kg^{-1}$), and Zn (23 $mg \cdot kg^{-1}$). Strontium (Sr) mineral showed a strong correlation, with a correlation copper of $r = 0.66$. The variety of mineral and protein levels seen in the examined cultivars is highly recommended as a valuable source of nutritious plant-based dietary resources, especially for developing concentrated protein sources. According to the findings, brown and red grain pericarp varieties have been identified as lucrative raw materials for investments in the industrial sector, mostly because of their high crude protein qualities.

Keywords: nitrogen, sorghum cultivars, soil, fertilizer, element content

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Introduction

Sorghum bicolor (L.) Moench is commonly cultivated due to its adaptability, nutritional value, resilience, and role as a subsistence crop in harsh environments (Mesfin and Girma, 2022). Agriculture and animal feed render it a crucial crop for ensuring food security and improving nutrition (Hossain et al., 2022). *Sorghum* is increasingly acknowledged as a useful commodity for enhancing global food security (Teferra, 2019). This study provides evidence for the simultaneous genetic improvement of sorghum varieties to boost their important mineral content (Ng'Uni et al., 2016).

Sorghum whole grain cultivars are mineral-rich plant raw (Pontieri et al., 2022). Ever since the advent of agriculture 10,000 years ago, wholegrains have formed a vital component of the human diet (Allai et al., 2022). Hidden hunger refers to many shortages in essential micronutrients, including iron, zinc, iodine, and vitamin A, despite sufficient intake of calories. Diets are high in calories but low in essential nutrients (Lowe, 2021). Whole grain items were used to mitigate the likelihood of non-communicable illnesses (NCDs) such as cardiovascular disease, malignancy, celiac disease, and type 2 diabetes (Nirmala and Joye, 2020). Inadequate intake of essential minerals in the diet may hinder cognitive and physical growth, reduce resilience to stress, and increase susceptibility to infections, particularly in children, pregnant women, and breastfeeding mothers (Paiva et al., 2017). The symptoms of mineral insufficiency vanish when adhering to a diet that includes supplements and compensates for the missing minerals required by the organs (Jimoh and Abdullahi, 2017). To guarantee sufficient consumption of all necessary amino acids, including a diverse range of protein sources in one's diet, is important. Legumes, almonds, and cereals are examples of plant-based protein sources (Leser, 2013). The estimation of crude protein in sorghum grains was shown to be correlated with the nitrogen content in sorghum grains (Nagy et al., 2023).

Further characterization of sorghum harvests is necessary to assess their food quality and other attributes, including the concentration of macro- and micro-elements (Pontieri et al., 2014). Moreover, concerning genotype variation, scientific studies have shown that the colour of the sorghum grain pericarp may be changed by genetic factors and environmental variables (Osman et al., 2022).

The study aimed to explore the level of crude protein and microelements such as Fe, Zn, Cu, and Sr in S. bicolor grain varieties cultivated under N fertilizer conditions, which can be the potential raw material for human consumption.

Material and methodology

Experimental construction

The field experiments were conducted at the Hungarian University of Agriculture and Life Sciences Research Institute at Karcag during the 2020 and 2021 seasons. The study involved six sorghum cultivars with diverse pericarp colours, including Zádor, ES Föehn, Alföldi 1, ES Albanus, Albita, and Farmsugro180. The impact of Calcic ammonium-nitrate (27% N CAN) fertilizer, applied at two rates (60 and 120 kg·ha^{-1}), was assessed on the respective soil types across the two seasons. This detailed methodology ensures the reliability and reproducibility of the study's results.

Seeding was conducted at the Karcag site in mid-May on 13 May 2020, by the prevailing weather conditions. The weeds were removed manually, and the harvest date was 15 October 2020. At the beginning of the growing season, the average rainfall was 17.8 mm, below the average of 54.2 mm. The temperature was also lower (14.6 °C) than the multiannual average of 16.6 °C, which delayed the initial emergence and development of the crop. The considerable rainfall in June and July has positively impacted the crop's development, especially before the 15 October 2020 harvest period.

Regarding the 2021 cultivation season, the sowing was carried out on 13 May 2021. The precipitation average (73.6 mm) was higher than the multiannual average (54.6 mm), resulting in a varied outcome. Concomitantly, the temperature (17.5 °C) was higher than the annual temperature (16.3 °C).

Dry matter measurements

The calculation of the dry matter content consisted of the following steps:

1. Drying step: The utilized oven was set at 130–135 °C, and the sample was dried until it was set up on a steady weight, ensuring we eliminated the moisture content from the tested samples.
2. Measuring the weight: After the oven-dried, we weighed the dry sample according to the standards outlined by (AOAC, 2016Hellevang, 1995). Moreover, by applying the desired formula for the dry weight:

$$\textit{dry matter content}\ (\%) = \frac{\textit{sample on a dry basis}}{\textit{sample on a moisture basis}} \times 100$$

Determination of crude protein

The nitrogen content of the sorghum grain samples was determined using the Kjeldahl method. A sample of 1 g was measured from the accessible zero point. The sample was transferred to a digestion tube with two S/3,5 Kjeltabs catalyst tablets and sulphuric acid (H_2SO_4), 14 mL as the desired volume. The tube was placed into a block heater set at 420–430 °C for two hours to break down the sorghum grain sample by oxidation. After the digestion process was completed after two hours, the samples were kept cool. When we calculated the nitrogen content of sorghum as a crude protein, a specific conversion factor of 6.25 was used according to (ISO 20483:2013, 2013) procedure.

Determination of mineral content

Based on the International Association for Cereal Science and Technology (2001) ICC Standards, Vienna. The samples were dried and milled with a Retsch SK-1 (Retsch GmbH Haan Germany) hammer mill using a sieve with 1 mm size. The grain samples from the experiments were analysed after wet digestion (Kovács et al., 1996) in the Central Chemical Laboratory of the Agricultural Center, University of Debrecen. To ensure the accuracy of the measurements, we used an authentic wheat sample marked BCR CRM 189 (whole grain). We participated in national (Wessling et al.) and international rounds (International Plant Exchange Network by Wageningen University) measurements. These instruments can simultaneously determine the contents of several elements, but the present paper concentrates on the Fe, Zn, Cu, and Sr elements. Concentration ($mg.kg^{-1}$) was expressed on a grain dry-weight basis.

Statistical analysis

Except as otherwise specified, all findings of the basic data were presented as the averages of three replicates. Statistical analysis was conducted using SPSS (Version 28.1). According to Shapiro-Wilks, the data followed the Gaussian distribution. Accordingly, the original data set was presented as mean ±SD, based on the varieties' and treatment variables' variances. Pearson correlation was created to examine the relationships between continuous variables. One sample test was used for the total protein examination.

Results and discussion

Nitrogen is an essential mineral nutrient for the development and growth of sorghum. It has the potential to increase crop yield by reducing leaching.

Variable responses to nitrogen fertilizer are observed among cultivars. Research endeavours examined the effects of nitrogen fertilization on the nutritional composition, protein content, and grain size of sorghum to advance the adoption of productive methods for sorghum farming (Khalfalla et al., 2024).

The results were evaluated based on the variation attributed to independent factors such as fertilizer and diverse cultivars (Bojtor et al., 2022; Khalfalla et al., 2024), which influenced the element concentrations, as shown in Table 1.

Macro-elements

Nitrogen (N)

The macro-elements were evaluated as crude protein (N) concentration, distribution, and variation, as indicated in Table 1 and Figure 1.

The variance showed a significant value of $p < 0.05$ based on the treatment variable, as demonstrated in Table 3. On the other hand, the crude protein shows a significant correlation between trace microelements, such as the correlation between the crude protein (N) and Sr among the different sorghum varieties (Figure 3). When plants receive a sufficient dosage of nitrogen, it can be reflected in the enhancing the bioavailability of the crude protein content in the crop grains (Izsáki and Németh, 2016; Khalfalla et al., 2024). Cultivar Zádor responded significantly to N fertilizer; the average was 13.5–17.5 $g \cdot kg^{-1}$, contrary

Table 1 The average values of the basic data for element concentrations in sorghum cultivars based on dry matter basis

Variables	Zádor	ES Föehn	Es Albanus	Alföldi 1	Albita	Farmsugro 180	P-value
N ($g \cdot kg^{-1}$)	15.3 ±4.0	15.8 ±1.80	12.3 ±1.40	16.5 ±1.70	11.9 ±1.90	18.7 ±2.40	0.01
Fe ($mg \cdot kg^{-1}$)	46.2 ±2.40	49.5 ±3.63	43.7 ±2.03	41.5 ±5.89	44.5 ±2.03	42.9 ±1.20	0.33
Zn ($mg \cdot kg^{-1}$)	22.9 ±0.93	27.1 ±2.01	23.08 ±0.44	24.7 ±1.20	23.61 ±0.72	24.8 ±7.30	0.02
Cu ($mg \cdot kg^{-1}$)	3.98 ±0.33	3.98 ±0.33	3.40 ±0.20	3.91 ±0.55	3.49 ±0.81	3.40 ±0.24	0.65
Sr ($mg \cdot kg^{-1}$)	1.2 ±0.07	0.9 ±0.08	0.9 ±0.08	1.0 ±0.04	1.9 ±0.18	1.6 ±0.03	0.05

Notes: All values demonstrated mean±SD, based on the three replications of the treatment (control and fertilizer)

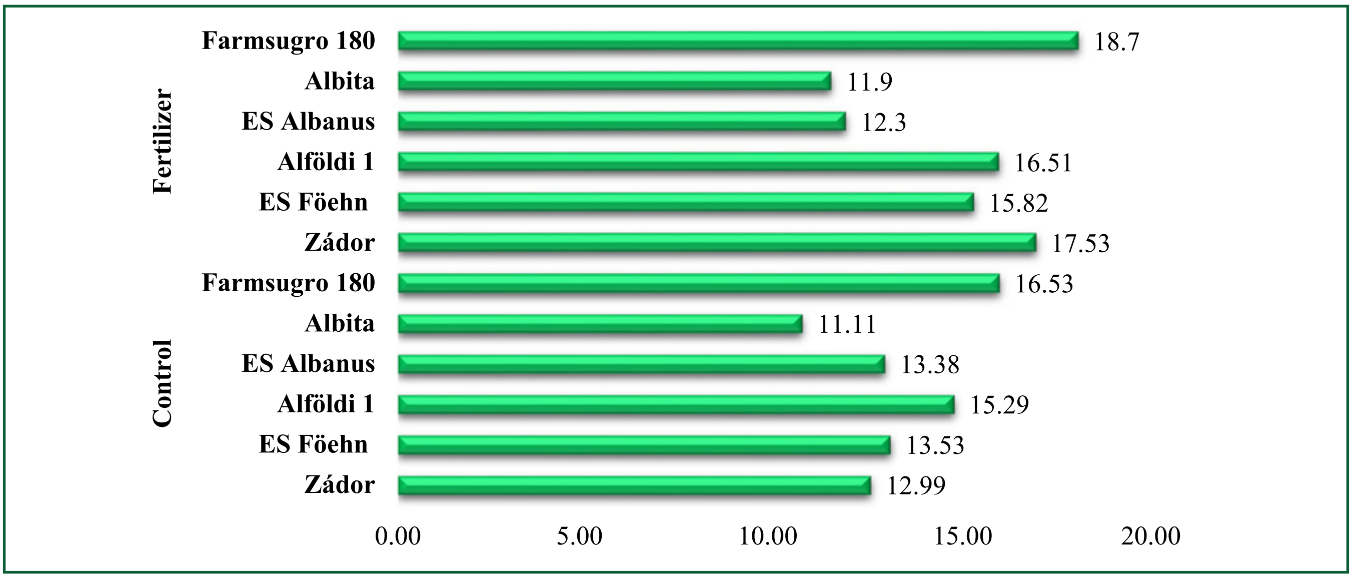

Figure 1 Crude protein concentration based on the treatment and cultivars
N indicates a significant value of p <0.05 in cases of treatment and cultivars

to the cultivar ES Albanus case, which had a reverse impact ranging from 13.4–12.3 g·kg^{-1} respectively. Sufficient application of N fertilizer positively correlates with protein and dry matter (Bojtor et al., 2022; Khalfalla et al., 2024).

Microelements

The concentration of microelements is crucial in inhibiting or accelerating the availability of several nutritional substances, including antioxidants, vitamins, and macro- and microelements (Kumar, 2021). The research provides clear evidence for the absorption and accumulation of microelements in several sorghum grain cultivars, as seen in Tables 1 and 2. Were recorded in the following manner:

Copper (Cu)

The previous research demonstrated additional advantages associated with the buildup of elevated

Table 2 Average of crude protein and trace microelement concentration based on the independent case of control and fertilizer

Cultivars	Elements				
	N (g·kg^{-1})	Zn (mg·kg^{-1})	Fe (mg·kg^{-1})	Cu (mg·kg^{-1})	Sr (mg·kg^{-1})
			Control		
Zádor	13.5	25.8	48.8	3.4	2.4
ES Föehn	13.5	23.0	41.1	3.4	2.7
ES Albanus	13.4	22.5	35.9	3.2	2.3
Alföldi 1	15.3	24.2	43.8	3.4	3.1
Albita	11.1	21.6	40.8	3.6	2.7
Farmsugro 180	16.5	23.7	38.8	3.6	1.4
			Fertiliser		
Zádor	17.5	22.5	48.1	3.5	1.8
ES Föehn	15.8	27.1	49.1	3.9	1.4
ES Albanus	12.3	23.4	43.7	3.4	2.2
Alföldi 1	16.5	24.6	45.8	3.5	2.2
Albita	16.5	23.4	44.5	3.5	2.1
Farmsugro 180	18.7	22.5	48.1	3.5	1.8

Notes: The demonstrated average values of element concentrations, based on the dry matter for the treatment case (control and fertilizer), indicate the variability of N fertilizer responses and interactions between the different sorghum cultivars

Table 3 Descriptive statistic of N (g·kg^{-1}) based treatments

N(g·kg^{-1})	Descriptive statistic		
	range	minimum	maximum
Control	5.8	11.1	16.5
Fertilizer	6.8	11.9	18.7

copper levels in sorghum tissues, due to its ability to absorb copper from the soil and accumulate into above-ground plant tissues, which can facilitate phytoremediation applications (Lima et al., 2019). Based on our research inquiry, we determined the appropriate level of Cu in the soil of the study site. Our findings indicate that using N fertilizer did not significantly impact Cu levels (Table 2). The concentrations of Cu varied from 3.4 to 3.5 mg·kg^{-1}, as measured by cultivar Zádor and shown in Table 2. Despite the substantial P value >0.05, the findings indicated that using N fertilizer benefits the copper (Cu) concentration.

Cu is the trace element with the lowest concentration among the analyzed element concentrations. The details of element accumulation in various sorghum cultivars were detailed in Figure 2; the result was linked to (Cui et al., 2022); the study showed that plants' copper and nitrogen assimilation, translocation, and distribution could interact effectively, as was evidenced in Figure 3, the correlation between Cu and Zn, r (5) = 66, P = 20.

Iron (Fe)

Iron is a vital element that plays a crucial role in several physiological functions, such as the transportation of oxygen, energy metabolism, and immune system functioning. As per the findings by (Singh et al., 2018; Chhikara et al., 2019), the use of nitrogen fertilizer was shown to have a beneficial impact on the iron content of the specific sorghum types. The current investigation results were consistent with the previous study, indicating a rise in the Fe concentration when comparing the control group to the group treated with fertilizer, as seen in Table 1 and Figure 2. The statistical test did not provide a statistically significant result, with a significant value of p >0.05. The investigated sorghum genotypes had the greatest microelement Fe content. The average concentration of Fe varied from 48.8 to 48.1 mg·kg^{-1}. The distribution of Fe concentrations was as follows: cultivar Zádor had the highest concentration, followed by cultivars Alföldi 1, Es Albanus, Albita, ES Föehn, and Farmsugro180, as is presented in Figure 2. Their changes demonstrated

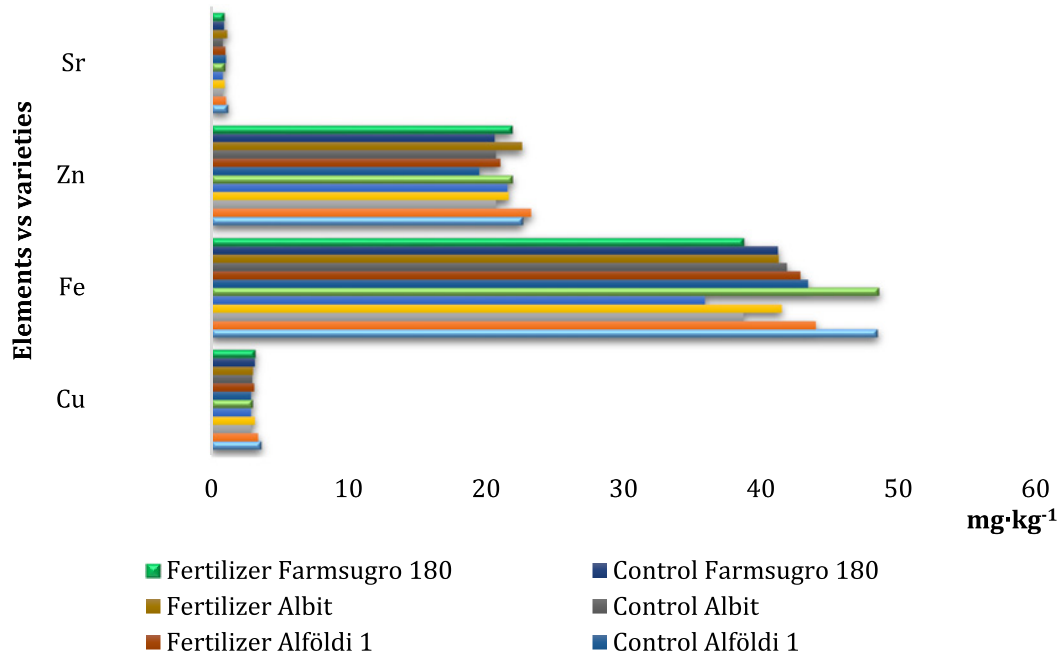

Figure 2 Microelement contents in the sorghum grain cultivars
*Values of an average of the elements based on dry matter calculation of three replications

a statistically significant value of p <0.035, as seen in Table 2. The result was lined (Khalfalla et al., 2024); the study presented the variety as a factor affecting iron mineral concentration in sorghum whole grains and processed grains.

Zinc (Zn)

Zinc is an elemental substance crucial in several physiological processes, such as growth and development. The concentration of micronutrients in sorghum grains may be affected by agronomic practices such as fertilization, irrigation, and intercropping (Gaddameedi et al., 2018). Zinc selectively activates the carbonic anhydrase enzyme (Tu et al., 2012). Nitrogen fertilizer had a mild impact on zinc (Zn) content, ranging from 19.5 to 23.3 mg·kg^{-1}. Table 3 demonstrates a statistically significant result with a P <0.05, as shown in Table 2.

The appropriate concentration of Zn was determined based on the investigation of microelements in the previous research, as shown in Table 1. The average total content varied from 21.6 to 27.1 mg·kg^{-1} across different sorghum cultivars. Moreover, the level of Fe was sequenced as follows: Zádor >Es Albanus >Albita >Farmsugro180 >ES Föehn >Alföldi 1. The zinc mineral ranges are shown in Table 2, with a significance value of (P <0.05).

According to Figure 3 Zinc (Zn) has shown a positive relationship with dry matter and copper (Cu). The correlation coefficient between Zn and N is 0.29. The correlation coefficient between Zn and Cu is 0.66; additionally, the correlation coefficient between Zn and Fe is 0.36 (Figure 3); the findings were linked to (Tripathi et al., 2010; Khalfalla et al., 2024), the research was discussed the factors can impact the zinc fortification strategy.

Strontium (Sr)

Sorghum plants are often grown on soils naturally rich in trace elements, such as strontium (Sr), which they can take up and accumulate in their grains. The distribution of Sr in the soil can vary widely, depending on the soil type and its geographical location, according to the result reported by (Shen et al., 2023). N fertilizer significantly impacted the uptake and distribution of strontium, as detailed in Tables 1 and 2. The average Sr was in a safe range of 1.4–3.1 mg·kg^{-1} respectively. Compared to the average in the previous studies, the concentration of Sr in soil ranged from 0.10 to 173 mg·kg^{-1}, with an average concentration of 11.9 mg·kg^{-1} (Shen et al., 2023).

The range of investigated Sr mg·kg^{-1} level was recorded by cultivar Zádor 2.4 mg·kg^{-1}, which was decreased to 1.8 mg·kg^{-1} after applying the N fertilizer dose. However, N fertilizer has no mentionable effect as a supplement for strontium biofortification in sorghum grains.

The strontium element accumulation varied depending on sorghum varieties; the lowest average of Sr

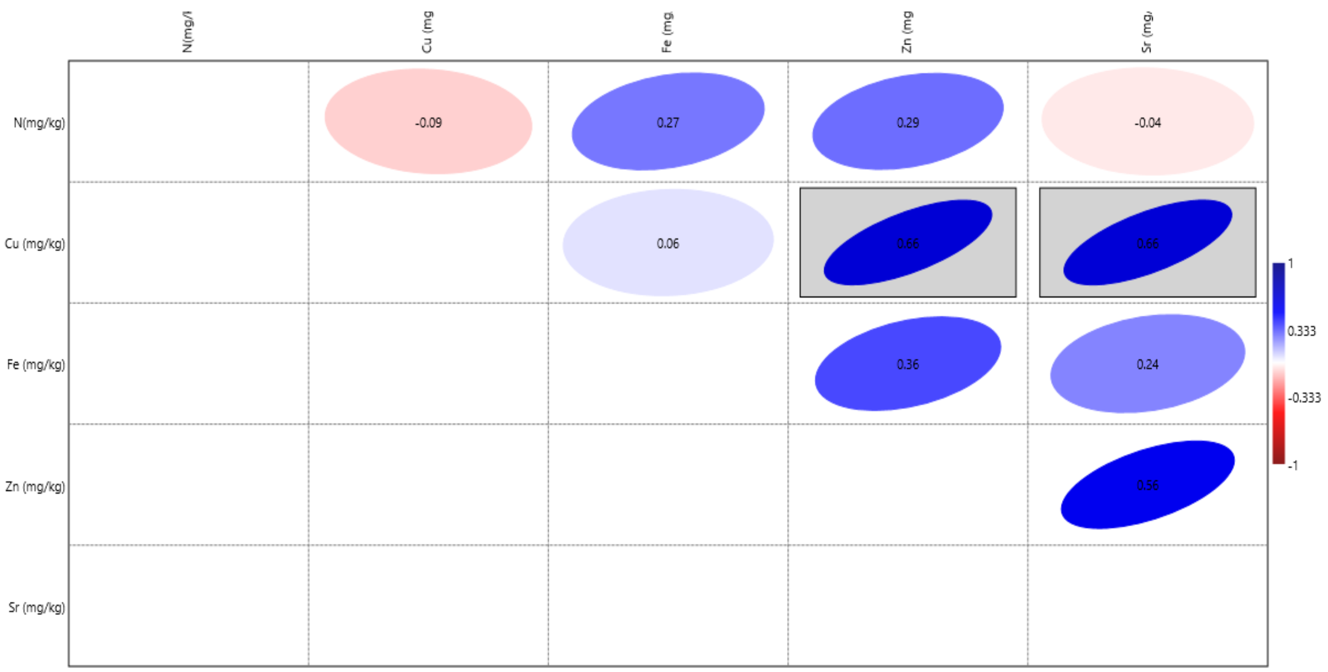

Figure 3 The correlation between nitrogen mineral and diverse microelement contents
Correlation between minerals presented values based on p <0.05

concentration was recorded by cultivars ES Föehn and Farmsugro180 after N fertilizer application, as shown in Table 1 and indicated by a significant value of $p < 0.05$, as shown in Table 1. The result of Sr distribution was identical to Qi et al. (2015); the distribution of stable strontium in the plant tissues varied depending on the cultivar and the crop species.

As detailed in Figure 3, nitrogen showed a strong correlation with strontium. Nitrogen proved to have a positive influence on microelement content. The element concentrations varied depending on the identical cultivars (Abdelhalim et al., 2019).

Conclusions

The study was conducted at Hungary's renowned sorghum cultivation location (Kacarg). In addition, the investigation assessed the nutritional composition of several strains of S. bicolor grain varieties, with a specific emphasis on their levels of crude protein and microelements such as iron (Fe), zinc (Zn), copper (Cu), and strontium (Sr). The reported findings can facilitate the understanding and general evaluation of the agricultural practice of the sorghum grain varieties of the common local cultivars in Hungary, specifically for industrial whole grain applications. In addition, respective grain cultivars, such as ES Föehn and Zádor, are promoted as superior local Hungarian cultivars according to their updated results of the high protein and microelement content, which can benefit the breeder to employ it as a benchmark for sorghum grain cultivars. The results provided current information on the analyzed cultivars, which might benefit breeders interested in developing sorghum grain for human consumption.

Conflict of interest

The authors have no conflicts of interest to declare.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

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